FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

NOVEMBER 30 - DECEMBER 1, 2004

DIMETHOATE: ISSUES RELATED TO HAZARD AND DOSE RESPONSE ASSESSMENT

TUESDAY, NOVEMBER 30, 2004

VOLUME I OF II

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1900 North Fort Myer Drive
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Reported by: Frances M. Freeman, Stenographer

C O N T E N T S

DR. ROBERTS: Welcome to the meeting of the FIFRA Scientific Advisory Panel. Today and tomorrow we'll be meeting on the topic of dimethoate, issues related to hazard and dose response assessment.

My name is Steve Roberts, and it's my pleasure to serve as Chair for this session.

I would like to begin by introducing the members of the panel that will be addressing this topic. And we'll go around the table and I would like to ask each member of the panel to indicate their name, affiliation and the expertise that they bring to this topic.

We'll start on my right with Dr. Ruby Reed.

DR. REED: My name is Ruby Reed from California Environmental Protection Agency. I'm a risk assessor. I do pesticide risk assessment. I also address controversial issues in risk assessments, some interesting issues of that. Also, I teach a class at UC Davis on risk assessment.

DR. RIVIERE: I'm Jim Riviere from North Carolina State University. My expertise areas are in pharmacokinetics and dermal absorption.

DR. FISCHER: I'm Larry Fischer from Michigan State University. I'm an environmental toxicologist with a special interest in biochemical toxicology.

DR. SLECHTA: I'm Deborah Cory-Slechta from the Environmental and Occupational Health Sciences Institute, which is an institute of the University of Medicine and Dentistry of New Jersey and Rutgers. My interest is in neurotoxicology, developmental and neurodegenerative diseases and also in behavioral toxicology.

DR. FOSTER: My name is Paul Foster. I'm from the National Institute of Environmental Health Sciences. I'm a reproductive and developmental toxicologist. My major research interests at the moment are effects of environmental agents on reproductive development.

DR. COLLINS: I'm Tom Collins. I'm with the Food and Drug Administration. I'm a developmental reproductive toxicologist with the Center for Food Safety and Nutrition.

DR. FRANCIS: I'm Bettina Francis. I'm at the University of Illinois. I'm a developmental toxicologist with a strong interest in pesticides. I teach a course in

pesticide toxicology. I have done research on organophosphates.

DR. BRIMIJOIN: I'm Steve Brimijoin. I'm a professor at the Department of Molecular Pharmacology at Mayo Clinic. I have a long interest in all aspects of the biology of cholinesterases and their toxicological implications.

MS. LEIN: I'm Pamela Lein with the Oregon Health and Science University Center for Research and Occupational Environmental Toxicology. My expertise is in the area of cell and molecular neurobiology, and I have a special interest, research interest in developmental neurotoxicology.

DR. PESSAH: I'm Isaac Pessah at the University of California at Davis. I'm a professor of toxicology. My expertise is in molecular and cellular toxicology.

DR. MACDONALD: Peter MacDonald, professor of mathematics and statistics at McMaster University in Canada with a general expertise in applied statistics.

DR. POPE: I'm Carey Pope. Professor of toxicology at the Center for Veterinary Health Sciences,

Oklahoma State University. I'm a neuro toxicologist.

DR. HARRY: I'm Jean Harry from the National Institute Environmental Health Sciences. My background expertise is in developmental and neurodegenerative diseases in toxicology.

DR. ISOM: I'm Gary Isom, neurotoxicologist from Purdue University. My area of interest is mechanisms of neurodegeneration.

DR. FREY: I'm Chris Frey from the Department of Civil Construction and Environmental Engineering at North Carolina State. My interests are exposure assessment and modeling techniques. I'm on the seven member SAP and I'm also the incoming president elect of the Society for Risk Analysis.

DR. HANDWERGER: I'm Stuart Handwerger. I'm professor of pediatrics and cell biology in the College of Medicine at the University of Cincinnati. I'm a pediatric endocrinologist. I do molecular and developmental endocrinology. My primary interest is the molecular mechanisms involved in human fetal growth and metabolism.

DR. CHAMBERS: I'm Jan Chambers from the College of Veterinary Medicine at Mississippi State University. I'm a pesticide toxicologist with emphasis on biochemical toxicology, metabolism and neurotoxicology.

DR. PORTIER: Ken Portier, statistician with the College of Agriculture, University of Florida, with interest in statistics in risk assessment.

DR. HEERINGA: Steve Heeringa, biostatistician with the Institute for Social Research at the University of Michigan. My area of specialty is in the design of population based research.

DR. ROBERTS: I'm Steve Roberts from the University of Florida. My expertise is in toxicology and risk assessment methodology.

I would like to point out that we have all seven members of the permanent panel in attendance today. We haven't had that in a long time, and that's great, including some of the new members of the panel that were added this year.

For those of you who are not aware, there is a permanent panel consisting of seven members, each

appointed for four year terms.

And while we represent a breadth of expertise as we discuss many of the technical issues that come before the panel, we need to expand that expertise by FQPA scientific advisory board members such as we have here today that have expertise on the particular topics that are being addressed.

The sessions are always chaired by a member of the permanent panel, but we rely very much on the expertise from scientists willing to come and attend these meetings and participate such as we have today.

As you can see, the SAP staff has assembled an outstanding panel with expertise in the topics that we are going to be talking about today.

I would like to turn the microphone over to Myrta Christian, the designated federal official for this meeting, because she has some announcements.

MS. CHRISTIAN: Thank you, Dr. Roberts.

Good morning. I am Myrta Christian. I will be serving as the designated federal official to the FIFRA Scientific Advisory Panel for this meeting. I want to

thank Dr. Roberts for agreeing to serve as chair of the FIFRA Scientific Advisory Panel for this meeting.

I also want to thank both the members of the panel and the public for attending this important meeting of the FIFRA SAP to review Dimethoate, Issues Related to Hazard and Dose Response Assessment.

We appreciate the time and effort of the panel members in preparing for this meeting, taking into account their busy schedules.

By way of background, the FIFRA SAP is a federal advisory committee that provides independent scientific peer review and advice to the agency on pesticides and pesticide related issues regarding the import of proposed regulatory actions on human health and the environment.

The FIFRA SAP only provides advice and recommendations to EPA. Decisionmaking and implementation authority remains with the agency.

As the DFO for this meeting, I serve as a liaison between the panel and the agency. I am also responsible for ensuring provisions of the Federal Advisory Committee Act are met.

As the designated federal official for this meeting, a critical responsibility is to work with appropriate agency officials to ensure that all appropriate ethic regulations are satisfied.

In that capacity, panel members are briefed with provisions of the federal conflict of interest laws.

In addition, each participate has filed a standard government financial disclosure report. I, along with our Deputy Ethic Officer for the Office of Prevention Pesticides and Toxic Substances, and in consultation with the Office of General Counsel, have reviewed these reports to ensure all ethic requirements are met.

For members of the public requesting time to make a public comment, please limit your comments to five minutes unless prior arrangements have been made.

For those that have not preregistered, please notify either myself or another member of the SAP staff if you are interested in making a comment.

There is a docket for this meeting. All background materials, questions posed to the panel by the agency and other documents related to this SAP meeting are

available in the docket.

Overheads will be available in a few days. Background documents are also available in the EPA web site. The agenda lists contact information for those documents.

At the conclusion of the meeting, the SAP will prepare a report as a response to questions posed by the agency, background materials, presentations and public comments.

The report serves as meeting minutes. We anticipate the meeting minutes will be completed in approximately six to eight weeks after the meeting.

Again, I wish to thank the panel for their participation, and I'm looking forward to both a challenging and interesting discussion for the next two days.

DR. ROBERTS: Thank you, Myrta.

I'm pleased to see we have present with us today for our session the Director of Office of Scientific Policy and Coordination for EPA, Mr. Joe Merenda. Good morning and welcome, Mr. Merenda.

MR. MERENDA: Thank you, Steve. It is my pleasure at this point at the beginning of the meeting to welcome all of the members, both of the permanent panel and the ad hoc members as well as members of the public to this session of the FIFRA scientific advisory panel.

Within EPA, the concept of having a transparent process for independent, external and rigorous review of our scientific products is very important to the way we carry out our activities.

And the FIFRA SAP is really the primary example within the Office of Prevention Pesticides and Toxic Substances for the way that we carry this out on the most significant issues that face us.

And we are very much appreciative of the expertise and effort that permanent members as well as ad hoc members of the SAP give to this activity. And we very much look forward to having a very spirited and effective discussion over the next two days on these important topics of Dimethoate.

Welcome, thank you and best wishes.

DR. ROBERTS: Thank you, Mr. Merenda.

I also would like to note that we have with us Dr. Randy Perfetti of the Health Effects Division Office of Pesticide Programs. Good morning.

DR. PERFETTI: Good morning, Dr. Roberts.

And echoing Joe's comments, I would like to welcome this panel for taking their valuable time to share their scientific expertise with us both today for this session and for the next two sessions. We have a, I believe, a very interesting week ahead of us.

These three sessions represent OPP's continuing attempt to use the latest methodologies and science for risk assessment. Today we're going to talk about using benchmark dose software for endpoint selection. Something that we believe is the wave of the future.

On Thursday, I believe, we're going to present a novel approach for using pharmacokinetic methodology to determine, to estimate exposures result -- pesticide exposures resulting from lawn treatments.

And finally, Friday, probably one of my favorite pet things is in a continuing effort to develop a PBPK model for cumulative risk assessment, we're going to talk

about some conceptual approaches to allowing outputs from the Lifeline software to be input into PBPK models so that we can continue the path down actually using a physiologically based pharmacokinetic models to do risk assessments.

Once again, I would like to welcome this panel and thank you very much for taking all your time to be with us this week. Dr. Roberts.

DR. ROBERTS: Well, all very interesting stuff, and I'm looking forward to discussing those subjects. It should be fascinating.

For our topic today, dimethoate, Dr. Diana Locke is going to begin the agency's presentations by providing us with some background. Welcome, Dr. Locke.

DR. LOCKE: Thank you. I'm Diana Locke, the risk assessor for dimethoate. I'm going to give you a brief history of the events that have transpired since 1999 and how they have brought us to where we are today.

In 1999, we presented a public technical briefing on dimethoate and the risk assessment based on the data and exposure models that were available to us at

the time.

Following the briefing in September of 1999, the agency issued the organophosphate data call in for all the organophosphates.

Then following in January 2001, we received the developmental neurotoxicity study as well as the accompanying range finding study and the companion comparative cholinesterase study.

Based on our analysis of the data at hand, the critical effects were determined to be increased postnatal pup mortality and cholinesterase inhibition.

In the spring of this year, Cheminova voluntary submitted a cross fostering study which was designed to explore the cause of decreased pup survival.

At that point, there were several toxicological issues at which there were diverse opinions. And the agency scheduled an SAP for July.

Just before the SAP, the health effects division received new data critical to the hazard assessment, and the SAP was rescheduled.

The new dimethoate data and information that we

received and reviewed were a one generation reproductive toxicity study, a new two generation reproductive tox study, a 28 day oral toxicity study.

All of these were conducted with cholinesterase measurements. And we received a benchmark dose analysis which was submitted by Cheminova.

The benchmark dose analysis indicated that cholinesterase, brain cholinesterase inhibition was the sensitive endpoint protective of pup mortality, which brings us to today's SAP meeting.

Today we bring several issues before the SAP for your consideration. We are seeking your input on the interpretation of pup mortality as a single dose effect or as a result of repeated dosing.

We're also seeking your input on the evaluation of the relationship between maternal toxicity and pup mortality and whether the data are sufficient to make this determination.

And lastly, we would like your input on the critical effect being brain cholinesterase inhibition and whether this effect is protective of pup mortality.

Though a small number of us sit here at the table, the extensive analysis and hard work that made this SAP possible were conducted by our much larger team. We would like to thank all of them and have them recognized today.

Our presentation team today consists of myself, and Cheryl Chaffey, the acting director of the Health Reevaluation Division of Canada's Pest Management Regulatory Agency, Kathleen Raffaele, who will be discussing the hazard assessment, Philip Villanueva, who will discuss the dose assessment, specifically the benchmark dose analyses, and, again, Kathleen who will integrate the hazard and dose response analyses.

And now I would like to introduce Cheryl Chaffey of the Pest Management Regulatory Agency in Canada.

DR. CHAFFEY: Good morning. My name is Cheryl Chaffey. I'm here today representing the Pest Management Regulatory Agency. For those of you who don't know, the PMRA is the Canadian counterpart to the EPA's Office of Pesticide Programs.

The reevaluation of dimethoate along with other

organophosphates was announced in Canada in 1999. Dimethoate has a similar use pattern in Canada to that found in the United States.

A preliminary hazard assessment of dimethoate was conducted by PMRA in 2002. Early discussions with EPA in 2002 revealed many similarities in our hazard assessments, and further collaboration in the dimethoate assessment was pursued.

Since 2002, the PMRA and OPP collaboration has included work sharing on the review of dimethoate and omethoate data, cross agency peer review, cross agency scientific discussion, culminating in today's presentation which represents a cooperative effort among agency scientists from both countries.

It is important to acknowledge that much of this collaboration would not have occurred without the registrant's consent to allow the exchange of information.

While my colleagues at EPA will be making the oral presentation today, the interpretation of the data presented reflects both the Canadian and American assessments to date.

The questions to the panel identify areas that warrant scientific debate in the view of both Canadian and American assessors.

The answers to these questions are relevant to furthering both the Canadian assessment as well as the American assessment of Dimethoate.

Perhaps what is noteworthy about the collective effort behind today's presentation is that it originated and emerged from scientific exchange among working level scientists rather than from a management directive.

One of the goals of the NAFTA technical working group on pesticides is to promote harmonization efforts within North America. Although much of the work sharing activity undertaken to date between EPA and PMRA has focused on new pesticides, the bilateral cooperation between our agencies on dimethoate very much embodies the spirit and goals of the NAFTA initiative.

On a personal level, it has been very satisfying to work with a high caliber of scientific professionals at EPA. They have demonstrated a culture of collaboration and openness and have embraced the participation of the

PMRA.

We hope that this collective effort and reevaluation activities will be continued in the future as we believe it effectively serves the needs of our many stakeholders.

I would like to now introduce the next presenter, Kathleen Raffaele, who will provide an overview of the hazard assessment of dimethoate.

DR. RAFFAELE: The next slide shows a little bit of a road map. You can see where we are. We have gone through the background information. Now I will be presenting the hazard assessment.

Phillip will then present the dose assessment and we'll go back to integrate the information in the final presentation.

As you all are aware, dimethoate is an organophosphate pesticide. Its metabolite, omethoate -- and the structure of it and its metabolite, omethoate, are shown on the slide above. The metabolite is actually the active compound which serves to inhibit acetylcholinesterase.

Although omethoate is not registered in the U.S., it is registered in several other countries. And, yes, there is some toxicity data available for omethoate as well as for dimethoate.

As Diana mentioned, two critical effects have been identified for dimethoate. Cholinesterase inhibition and increases in pup mortality.

Cholinesterase inhibition is an effect which is common with many other organophosphate pesticides. In this presentation, we'll focus on the brain cholinesterase inhibition which occurs for dimethoate at doses similar to or lower than inhibition in other compartments.

Pup mortality, which was also seen following administration of dimethoate to maternal animals, is an endpoint which is not common to other organophosphate pesticides, but it has been seen in multiple studies with dimethoate.

In this presentation I will first describe the design of the relevant submitted studies, then briefly review the results of the studies and compare the findings across the different studies, including some of the

limitations in these comparisons.

The next slide is a diagram of the study design for the main developmental neurotoxicity study which was submitted for dimethoate.

As Diana mentioned, this study was conducted as part of a data call in for the organophosphate pesticides. And the data call in specified that DNT studies be conducted according to the OPPTS guideline with a few additions. That included extending the dosing from postnatal day 10 to postnatal day 21, evaluating the adequacy of dosing to the pups and also providing information regarding comparative cholinesterase inhibition in young and adult animals.

I will briefly review the study design which hopefully many of you are familiar with. In the DNT study, dosing is -- and particularly this study, gavage dosing to the dams was begun on gestation day six and continued through postnatal day 10.

Starting on postnatal day 11, offspring were dosed again by gavage through postnatal day 21. During the period of lactation and right around weaning and again

at postnatal day 60, a variety of behavioral assessments were conducted.

However, these are not at issue in our discussion today. So results of those won't be discussed further here. The doses for the dimethoate study were 0.1, .5 and 3 milligrams per kilogram. And the group size was approximately 23 to 24 animals per group.

This information will be provided on a summary slide later, but just for your information as we go along.

In addition to the main DNT study, a range finding study was submitted. This study was conducted at slightly different doses and the purpose was to determine the doses to be used in the main study.

The design was similar to that of the main study with dosing to the dams starting on gestation day 6 continuing through postnatal day 10 followed by direct dosing to the offspring.

In this study, cholinesterase evaluations were done as well as evaluating just the basic toxicity. No other behavioral evaluations were done.

Smaller number groups sizes were used in this

study of eight to 10 per group. Slightly different doses of 0, .2, 3 and 6 milligrams per kilogram were administered.

As part of the data call in, data on comparative cholinesterase were also required. The comparative cholinesterase study was conducted as a separate companion study to the main DNT study. And consists of two parts.

In the first part, acute exposure to a single dose was assessed in both postnatal day 11 offspring and in adults following a single dose of dimethoate at the same dose as used in the main DNT study.

In addition, cholinesterase inhibition was assessed following repeated dosing according to the design as you see in the slide. Again, the maternal dosing was similar to that in the main cholinesterase study and the doses were the same from gestation day six to postnatal day 11. And then direct dosing was administered to the pups from postnatal day 11 to 21.

Cholinesterase was assessed in dams and fetuses on gestation day 20, pups on postnatal day four prior to the start of direct dosing. And again in pups on

postnatal day 21 after 11 direct exposures had been received.

In addition to provide a direct comparison following a similar number of dosing, adults were dosed for 11 days, both males and females, and assessed for cholinesterase inhibition as well.

As Diana mentioned, the registrant recently conducted a cross fostering study to further evaluate the question of whether the mortality seen in offspring could be attributed to maternal toxicity.

The design of this study is presented on the next slide. It is a little more complicated. I'll go through it hopefully in a way that will make sense.

The dosing was similar to that used in the DNT study in that it was direct gavage to the dams starting on gestation day 6 and continuing through postnatal day 10.

However, pups were cross fostered at birth to mothers receiving different doses or similar doses to their parents. Two doses were used. 3 and 6 milligrams per kilogram and the study consisted of six dose groups.

Two groups were continually receiving the same

dose, both prenatal and postnatally. One control group and one at the highest dose, 6 milligrams per kilogram.

In addition, two groups of animals that had been initially exposed in utero to control only were cross fostered to dams receiving either 3 or 6 milligrams per kilogram.

In addition, there were groups that had been exposed in utero to either 3 or 6 milligrams per kilogram that were cross fostered to dams receiving control only. And the doses -- you can also see the various dose groups here.

In addition -- cholinesterase was not evaluated in this study and the sample size was similar to that in the main DNT study, 22 to 25 animals per group.

So the sensitivity of this study for evaluating pup mortality should be similar to that in the main DNT study.

In addition, detailed maternal observations were gathered in this study to provide more information regarding toxicity to the dams.

In addition to the DNT and related studies which

were conducted using gavage dosing, we have several studies available which were conducted according to the reproductive toxicity guideline.

In these studies, exposure is slightly different, and usually the test compound is administered by mixing it with the diet.

Usually, the dosing starts -- the dosing regimen is also considerably different and starts much earlier, so that animals are usually dosed approximately 10 weeks prior to the start of mating. Dosing then continues through mating, gestation and lactation.

At the end of lactation, pups for the second generation are selected and dosed again prior to mating, during mating, gestation, and lactation. You can continue for additional generations according to the design in the study.

We have two reproductive toxicity studies available with dimethoate that were continued for two generations and additional range finding study that was continued for one generation only.

In addition, we have two reproductive toxicity

studies with omethoate, one which was conducted by mixing the test substance with the diet and one which was conducted in drinking water.

In all of these studies, cholinesterase evaluations were done at various time points, mostly in adults prior to sacrifice.

Since these studies also provide information about offspring survival and cholinesterase inhibition, we thought they were useful to compare the results with that in the main DNT study.

The next slide I'm not going to talk about very much. You probably can't read it, most of you, but it is available -- there is a bigger copy available to everyone. What it does is provide the basic information on all of the studies we're discussing on one page.

You can see the doses and which parameters were measured in each study. It is available mainly for your reference as part of the discussion. There are also hard copies available for the public if people need a copy.

Now I'm going to briefly describe the results of each study. For all the studies, I would like to remind

you we're discussing only the results which are related to the issues we're presenting to the SAP, mainly brain cholinesterase inhibition, maternal toxicity and the increase in pup mortality.

As you are all aware, full reports of these studies were provided in the docket.

The reason we're focusing on brain cholinesterase and not the other compartments is that it has found to be a critical effect with dimethoate occurring at doses similar to that in the blood compartments.

Data on the other compartments is available in the study reviews which were provided to the panel and in the docket.

In the main DNT study, as I mentioned, cholinesterase inhibition was not measured. However, the information are available from the companion study.

There was a dose related increase in pup mortality seen in this study with a no effect level of .1 milligram per kilogram and the increase in dose starting at .5 milligrams per kilogram.

This effect was seen only during early lactation prior to the start of direct dosing. There was no increase in death during the direct dosing period of the pups.

The increase was represented mostly as an increase in the number of total deaths, not in the number of litters in which death seemed to occur.

The results were a slight increase in total litter loss seen mainly at the highest dose. There was no indication of maternal toxicity in this study, but the maternal assessments were limited and consisted only of clinical observations.

The next slide lists the measurements by which I'm going to show you in the following slide. There are four different measures of pup mortality that we looked at, total litter loss, which could have occurred any time between postnatal day 1 and 21. The data in the slide will show the number of litters with at least one death from postnatal day 1 through 4 only, since that was the time when the greatest number of deaths occurred, and also the actual total number of individual pups that died

during that same period.

The graph will also present the mean number of dead pups per litter again for the entire lactation period of postnatal day 1 to 21.

And you can see these are the results of the main DNT study. The first figure shows the total litter loss. And you can see basically all of these show the same response looking at each measure.

You can see that there is no difference between the control and the low dose group. But there is an increase in the two higher dose groups. That's less apparent and probably not as -- probably not actually an increase in the number of litters at the mid dose, only at the high dose.

But then when you look at the total number of deaths from postnatal day 1 to 4, there is a clear increase, and also when you look at the postnatal day 1 to 21 group.

It should be important to note that the scales are different on these. And obviously, since we're switching days, the denominators are slightly different

for the different figures.

Next, we'll present the results of the range finding study. The range finding study, both cholinesterase and pup mortality, can be evaluated. So first I will present the results for the brain cholinesterase.

There was a dose related increase in brain cholinesterase inhibition seen at both 3 and 6. Remember, the doses are slightly different for this study.

It was seen in fetuses, pups, and dams. Again, it is important to remember there is a smaller number of animals in this study which is eight to 10 per group. This slide shows the dose response curve for the cholinesterase inhibition. And you can see there is the dose related decrease.

It is also important to note that the inhibition in the dams shown here is greater than that which was seen in the fetuses, and this shows the two sexes, both of which had basically similar results.

There is also an increase in pup mortality in the range finding study as measured by individual. Looking

at the total number of individuals that died as well as total litter loss, this increase was seen only at the highest dose in that study.

At that same dose, there was a slight decrease in pup body weight, as well as slight decreases in maternal weight gain.

Again, the same four measures are going to be presented for the data from the range finding study. You can see very clearly that there is an increase only at the highest dose in this study, again, which looks very similar by the different four measures.

The companion DNT range finding study consisted of similar number of animals per group as the range finding study with 10 animals at each dose.

There was again a dose related increase in brain cholinesterase inhibition following the repeated dosing at the mid dose and the high dose for all the groups that were evaluated.

There was no increase in pup mortality in doses up to 3 milligram per kilogram in this study. So I have not made a separate slide to present that data. The next

slide shows the results of the cholinesterase evaluation.

This slide shows the inhibition levels for the dams, the fetuses and also the evaluation for pups on postnatal day 4. You can see again the dams show the most inhibition with less inhibition in the fetuses and a very small level of inhibition in the postnatal day 4 animals.

This might lead us to believe there was probably not very much exposure during lactation. This was taken again prior to the start of the direct dosing.

This shows the results of the repeated dosing. These were the evaluations done at postnatal day 21 in males and females pups, as well as after 11 consecutive doses in adults.

You can see basically the curves are all lying on top of each other. There doesn't appear to be any difference in the level of inhibition seen at the two different ages.

It is important to remember that the exposure for the pups was slightly different than that for the adults and that these pups were also exposed in utero and potentially during lactation before the start of direct

dosing.

The next slide shows the results of the cross fostering study. Again, this study was conducted similar to the DNT study using gavage dosing. But was continued only through postnatal day 11 since that was the period during which the most deaths were seen in the main DNT study.

The ends were similar to the DNT study with 23 to 25 animals per group, and, again, cholinesterase was not evaluated in this study.

This study shows a clear increase in pup mortality at the high dose of 6 milligrams per kilogram, regardless of the treatment scenario, whether the treatment consisted of prenatal only, postnatal only, or continuing exposure to the dams.

Although we did note there seemed to be an earlier death -- the deaths seemed to occur earlier in those animals exposed only prenatally than in those exposed only postnatally.

The effects of 3 milligrams per kilogram were equivocal with a slight increase perhaps from days 5 to

11. This was probably not above the normal expected background.

We would also like you remind you that at 3 milligrams per kilogram there was no group with continuing exposure throughout pre and postnatal period, but only separate prenatal or postnatal groups.

There was an indication of maternal toxicity in this study manifested mainly as an increased incidence of restlessness and scattering of the pups. This was associated with postnatal treatment groups at both doses.

The next slide shows for this study there was no total litter loss. We're presenting in the graphs for only three measures. And I also would like to remind you that the numbers for some of them are postnatal day 1 to 4 only. And since in this study some of the deaths occurred later, they will look slightly different for the postnatal day 1 to 4 versus the PND 1-11 timeframe.

Since this is a little more complicated, I'm going to go through which bar is which.

The first bar for each graph is the controls which were exposed only to vehicle throughout the study.

The second two are those receiving prenatal only exposures at 3 and 6 milligrams per kilogram.

The next two bars are those receiving postnatal exposure. Again, this is exposure to the dam, not to the pup during gestation -- up to gestation day 11.

The last bar is those receiving exposures both pre and postnatally at the highest dose of 6. These two top ones show data from the postnatal day 1 to 4 only period. This shows data from days, postnatal days 1 through 11.

You can see looking at that basically there is no difference at this time point in anything except the group receiving combined exposures.

And here there is an increase in the total number of deaths for those receiving only prenatal exposure at the highest dose.

This shows a slight dose relationship, but, again, this difference is very small at 3 milligrams per kilogram, which seems to be the same regardless of the pre versus the postnatal exposure.

There is a slight increase at 6 milligrams for

the prenatal only group and for the postnatal only, again, with the most deaths occurring in that receiving the combined exposure.

I'll just describe briefly the results of the reproductive toxicity studies for dimethoate and omethoate to remind you again of the differences in the duration and amount of administration with these studies which were conducted by continuous dietary administration.

Because of this, the doses are really not directly comparable with the gavage doses used in the DNT study and cross fostering study.

In addition, there was a decrease in fertility seen in some studies. And since fewer pups were born, it is not really possible to compare the number of pups deaths directly again with those in the main DNT study where there were no issues regarding fertility.

With these caveats, there was a difference in the impact of pup survival depending on the studies. There was no increase in pup mortality seen in the two two generation studies was dimethoate. And these included dietary doses up to 6 milligrams per kilogram.

However, there was an increase in pup mortality seen in the one generation reproduction range finding study at doses of 5.8 or 7.5 milligrams per kilogram.

In the two omethoate studies, there was a slight increase in pup mortality, but it was not consistent across the generation. It was seen in some matings, but not in other matings.

Cholinesterase data for these studies which was available mainly for adults showed decreases in brain cholinesterase activity at doses of 1 milligram per kilogram or higher.

Data for offspring in these studies are limited. There was a little data from postnatal day 4 which basically agreed with the data from the DNT study that there really wasn't much -- didn't look like there was much lactational exposure.

But mostly, since there was no direct dosing to the pups in any of these studies, we really don't have any that allows to compare the age related sensitivity.

Based on these results, we felt that the reproductive studies support the pup effects in that there

was an increase in mortality seen in some of these studies. However, the doses associated with that increase seem to vary among the different studies.

And this is another graph, another table available for your reference which includes the doses at which various effects were seen again by listing all the studies and including the dose information.

The next slide just summarizes the findings for pup mortality. There was an increase in pup mortality in the main DNT study, the cross fostering study, the DNT range finding study, and the one generation reproductive toxicity study with dimethoate.

The results of the reproductive toxicity studies generally supported the findings from the DNT study, although there were differences among the studies in the dose at which the response was seen. And it was clear that the main DNT study found the increased pup mortality at a much lower dose than the other studies.

For cholinesterase inhibition, we have data available from multiple studies. And in general, the brain compartment was found to be the most sensitive.

Based on the data available from the companion cholinesterase study, there was no indication of an increase in sensitivity associated with the age of the animals.

That concludes this portion of the presentation.

DR. ROBERTS: Thank you, Dr. Raffaele, for a very nice summary of the studies. Let me give the panel the opportunity to ask any questions if there is anything they are unclear about regarding these studies.

Yes, Dr. Harry.

DR. HARRY: I have two specific questions. One was when you were doing the cholinesterase measurements, what was the average variance? There is no --

DR. RAFFAELE: For the brain, it was pretty tight. The information is available. In the background paper, the standard deviations were included. But for the brain in general it was very tight. We actually did see significant effects with very small changes.

DR. HARRY: The other one I know when you are digging in the background document, but just to sort of bring it up, when you were looking at the pup mortality,

you are looking at the mean.

But if I remember correctly when I was looking back, there were a few litters that were sort of driving that effect.

DR. RAFFAELE: There did seem to be difference among the different litters in that some of them had much larger number of pups that died than others did.

DR. HARRY: Thank you.

DR. ROBERTS: Dr. Foster.

DR. FOSTER: I wasn't clear when I read through the background document. Did you do any of your statistical analysis where you looked at individual pup death but actually nested it by litter?

DR. RAFFAELE: Phillip is going to talk about the BMD analysis that we did and it did include nesting.

DR. FOSTER: I was actually thinking more about when you want to get -- trying to get litter means and so on and so forth out of this.

You can just nest the individual data by litter.

DR. RAFFAELE: No, we didn't do any additional analyses with that.

DR. ROBERTS: Dr. Harry, then Dr. Isom.

DR. HARRY: Just one more quick one.

In the DNT study, it is usually the litters are culled down to a certain size.

DR. RAFFAELE: Yes, in the DNT the litters were culled. They were not culled in the cross fostering study.

DR. HARRY: That's what I thought I noticed when I read. Did you look at whether litter size influenced death when you did the cross fostering study?

DR. RAFFAELE: I didn't look at that. That's a good question.

DR. HARRY: Then the other one. For the reproductive, the two generation studies many times that is not culled.

DR. RAFFAELE: I think all these were culled.

DR. HARRY: Same size as the DNT?

DR. RAFFAELE: I believe so. I can check and make sure, but generally they are usually culled to four or six or eight.

DR. ROBERTS: Dr. Isom.

DR. ISOM: In the main DNT study, cholinesterase inhibition wasn't measured, I guess, activity. But it was stated that maternal toxicity was not seen.

What kind of indexes were measured for maternal toxicity at that point?

DR. RAFFAELE: There are some detailed clinical observations that are specific to the DNT study, but really the observations are not much more detailed than just the regular clinical observations that you will see.

In the DNT studies, they actually take the animals out of the cage and evaluate them, but for the most part they are just looking at them checking for the normal cholinergic signs.

They may watch them walk around in the open field a little bit, but they are very -- not very detailed observations.

Certainly it seems that cholinesterase inhibition was inhibited at those doses and so it is possible there could have been signs that were missed.

DR. ROBERTS: Yes, Dr. Collins.

DR. COLLINS: In your culling, was the culling

done randomly in these studies.

DR. RAFFAELE: Yes, the culling is done randomly.

DR. ROBERTS: Dr. Pope, then Dr. Lein.

DR. POPE: I was wondering what kinds of balancing is done in the design of the DNT studies, balancing between litters as far as dosing or is there any balancing at all.

DR. RAFFAELE: I'm not sure I'm understanding what you are asking.

DR. POPE: For example, I don't know much about the DNT study, but if I was doing a study where there were pups, I would be trying to cross balance between litters for different doses. What kind of considerations are --

DR. RAFFAELE: All the doses -- I'm not sure I'm still answering, but I will try and see. All the pups from a specific litter would have been dosed the same.

So the litters are assigned to a dose group and the dosing is either to the mom or to the pups -- for that litter would all be the same. Was that your question?

DR. POPE: I suppose so. After the pups begin

to be directly dosed, they continued to be dosed in the same way?

DR. RAFFAELE: At the same dose that the moms had been dosed.

DR. POPE: There is no balancing.

DR. RAFFAELE: There is no within litter balancing, but then there is different litters for the different doses.

DR. ROBERTS: Dr. Lein.

DR. LEIN: I was just curious. One of the issues in the cross fostering appears to be whether you include the pups that are stillborn and die on postnatal day 1 in your analysis. And what is the EPA's rationale for including those pups in their analysis?

DR. RAFFAELE: I guess we felt that if we -- those data were excluded, it was really under representing the number of pups that had died. In the regular study, all the pups that died would be included for all the groups.

So we felt to exclude those pups we would really lose information.

DR. ROBERTS: Dr. Foster.

DR. FOSTER: I had one more and that was on the cross fostering study. The more I looked at that, I couldn't see in the control litters the actually, I suppose, or exposed as controls both through in gestation and lactation, that they were actually cross fostered, the pups from the different dams.

DR. RAFFAELE: Right. The control pups were not cross fostered. And the ones that were treated at the high dose, both pre and postnatally, were also not cross fostered. So there was no control just for the cross fostering.

DR. ROBERTS: Any other questions.

If not, let's go ahead and listen to the presentation on the benchmark dose analysis.

Mr. Villanueva, are you ready to go?

DR. VILLANUEVA: Good morning. My name is Philip Villanueva. My title is mathematical statistician. My background is applied mathematics. I have a Master's degree. I'm with the Health Effects Division.

And my part of the dimethoate team was to

perform benchmark dose analyses for the two endpoints, brain cholinesterase and pup mortality that I'll be talking about today.

Again, we're going to go back to the road map here. We have already discussed the background and the hazard assessment. I'll continue with the dose assessment and Dr. Raffaele will follow up with an integration of both of these facets.

Generally, a benchmark dose analysis consists of fitting mathematical models to the dose response curve that's observed from the responses of the experimental dose groups.

And a benchmark dose refers to the dose which is expected to elicit a specific response. And this response is generally referred to as the benchmark response. And depending on the endpoint being selected, you can specify that benchmark response to be at various levels.

The benchmark, the BMDL, as it is commonly called, is the lower 95 percent confidence limit on the benchmark dose.

Generally, there are two approaches for

evaluating the observed dose response relationship seen in the toxicity studies. The first is the NOAEL/LOAEL approach, which is basically limited to making comparisons between the observed responses of the experimental doses only.

One of the advantages of the BMD approach is that it allows one to compare the expected responses at any dose within the experimental dose range.

We consider benchmark dosing to be the more appropriate method for comparing endpoints across ages and studies. Generally, benchmark dose approach is not as limited to the experimental doses as the NOAEL/LOAEL approach.

And ultimately, at the end what we would like to do is compare the BMDLs for the various endpoints in this case, cholinesterase inhibition and pup mortality.

For the selection of the benchmark responses, we determined that the brain compartment was the most sensitive to dimethoate cholinesterase levels. I believe the others available were red blood cell and plasma. I think that's correct.

For cholinesterase inhibition, both the BMD and BMDLs were compared for the various studies. And we selected 10 percent as the benchmark response. So a 10 percent change in the background cholinesterase level.

From a prior statistical analysis, it was determined that 10 percent was about as much as can be determined from the various toxicity studies in general.

Also, for pup mortality, BMDs and BMDLs were calculated. Five percent was used. A five percent extra risk was determined to be the benchmark response, which is routinely done for any quantal developmental endpoint.

For pup mortality, the benchmark dose software developed by our Office of Research and Development was used to determine the benchmark doses. The BMDS has been both externally and internally peer reviewed.

For brain cholinesterase inhibition, we used a decreasing exponential model that has been endorsed by -- it was endorsed by the SAP in 2001. Generally, this decreasing exponential model is not included in the benchmark dose software, but will be eventually incorporated.

Pup mortality data were obtained from the dimethoate DNT study, the main DNT. There were two study periods that were examined, PND 1 through 4, post natal day 5 through 11. These were analyzed separately due to the culling event that reduced the litter sizes I believe to eight pups per litter.

For pup mortality, we used the BMDs nested dichotomous models. Those are the Nlogistic model, also known as the nested log logistic model, NCTR model and RaiVR model. For each of these models, an intra litter correlation was taken into account and the likelihood function. Thus, being termed nested dichotomous models.

For each of the -- with each of the models, the BMD 5 and BMD 10s and also their corresponding lower limits were calculated.

And they correspond to an extra 5 and 10 percent risk of pup mortality compared to the background incident respectively. Also, up there we have the formula for extra risks for those that are interested.

Basically, the probability of pup mortality at the benchmark dose, the difference between that and the

probability of mortality at the background is divided by the probability of there not being any incidence.

Here are the results for the three models for both of the study periods, postnatal day 1 through 4 and PND 5 through 11. For all the models, there was no litter covariate selected.

As you can see, these are the benchmark dose 5 values for each of the three models. They are all within the corresponding dose ranges. And also we can see here that the AICs are very similar, the AIC being a parameter, a statistic that's used to select models that are using the same data set based on, in part, the fit of the model and the numbers of parameters used to specify the benchmark dose.

The Nlogistic model resulted in slightly smaller BMD values. And since the AICs were all very similar for the three models, the benchmark dose values from the Nlogistic model were selected for comparison later on with the brain cholinesterase BMD values.

The formula for the Nlogistic model is also given on this slide. Here we have a gamma representing

the background response level and the alpha and beta representing the intercept and slope parameters that in part determine the shape and location of the logistic distribution incorporated in the model.

Here we have a graph of the model results. The red actually symbolizes the probability of response at the various doses. We also have the responses of the various dose groups.

Here the BMD 5 for postnatal day 1 through 4 was determined to be .5 milligrams per kilogram. The 95 percent lower limit of the BMD 5 is .3 milligrams per kilogram.

For the postnatal day period 5 through 11, all of the model results had BMD 5s that were higher than those. And the postnatal day 1 through 4s can be seen here.

Again, these are the smaller BMD values from the three models. In this case, it is the NCTR model with results of a BMD of 1.7 milligrams per kilogram and a BMDL 5 of .8 milligrams per kilogram.

Next, brain cholinesterase data were obtained

from the following dimethoate toxicity studies. We have the comparative cholinesterase study, the DNT range finding study, the one generation study and two 2 generation studies, one being done I believe in 1992 and the other in 2003, I think. There is also a 28 day dietary toxicity study.

As I mentioned before, brain cholinesterase model was -- brain cholinesterase inhibition was modeled using the basic decreasing exponential function that was endorsed by the SAP.

This model in particular does not include the low dose shoulder of the expanded model, although, there hasn't been any evidence that a low dose shoulder needs to be modeled for the dimethoate, for dimethoate in particular, with respect to brain cholinesterase.

The BMD 10s values were calculated for the various toxicity studies. Again, BMD 10 represents a 10 percent change in the background level of cholinesterase activity.

Here we have the formula, the difference between the brain cholinesterase values at the control and the --

at the control and the benchmark dose being 10 percent.

Here is the formula for the decreasing exponential model where Y is the cholinesterase activity. M is the dose scaling factor. And we have A being the background cholinesterase activity. B is the limiting high dose cholinesterase activity, so the maximum amount that we would expect brain cholinesterase to reach.

We looked at repeated dosing and acute -- repeated dosing and single dosing data. For the repeated exposures, we saw a clear dose response relationship.

Also, most of the models, with few exceptions, obtained adequate goodness of fit statistics resulting in P values of generally greater than .0 5.

Benchmark dose 10 values were all within their respective dose ranges with the exception of the postnatal day 4 group from the comparative cholinesterase study.

Also, as we'll see in the next couple slides, the BMD 10 values were similar across the various routes of administration and the ages and sex, except, of course, for the postnatal day 4 group, which, as I mentioned before, produced BMD values outside the experimental dose

range.

Here we have the various studies highlighted with their MRID numbers and the subpopulations.

As I mentioned before, the P values from the goodness of fit statistics are generally adequate with few exceptions here.

Also, you can see that the BMDL values are very similar for most of the studies except in this case, as I mentioned, the postnatal day 4 offspring.

I believe the highest dose was 3 milligrams per kilogram. You can see that they result in BMD values outside of the experimental dose range.

Next, we have a graph of the BMD values and their corresponding lower limits. You can see again that the PND 4 values are well above the other BMD values that were estimated for the various subpopulations.

As you can see, they all are fairly consistent across the different subpopulations and routes of administration.

For the acute dosing data, there was also a fairly clear dose response relationship. Again, adequate

goodness of fit was obtained for the decreasing exponential model.

And the various subpopulations produced similar BMD 10 values. And, of course, they were within their respective dose ranges.

We don't have nearly as much data for the acute dosing as we do the repeated dosing.

Here we have adults and offspring. And they are fairly similar with the BMD 10 values for acute dosing as expected being larger than those for repeated dosing. And the P values again for the goodness of fit statistics are adequate.

For the repeated exposure, the range of the BMD 10 values were .2 to 1 milligrams per kilogram with the exception of the postnatal day 4 group. The range of the BMDL 10s were .2 to .8.

Here I have a graph of the model that was fit to the group from the DNT range finding study. These are the gestational day 20 dams. This is the subpopulation that resulted in the largest BMD and BMDL 10 values.

From the 28 dietary study, we have the day 28

adult males that resulted in the largest BMD 10 values.

Again, for the acute exposure, the ranges are 1.5 to 2.6 milligrams per kilograms for the BMD 10s. And for the BMDL 10s, the range is 1.3 to 2 milligrams per kilogram.

Again, these are graphs of the highest and lowest BMD 10 and BMDL 10 values corresponding to the postnatal day 11 females and the day one adult males, both from the comparative cholinesterase studies.

And that concludes my presentation.

DR. ROBERTS: Thank you. Let me ask the panel members if they have any questions. Dr. Harry.

DR. HARRY: Forgive me if I missed this, but you did -- all of your analysis that you did for the cholinesterase endpoints was across all studies that you had the data. Correct?

So it was the reproductive studies, the generation 2 gen studies and that type of thing?

MR. VILLANUEVA: Correct.

DR. HARRY: The pup mortality data, you limited that evaluation to the developmental neuro tox study?

MR. VILLANUEVA: Correct.

DR. ROBERTS: Dr. Foster, did you have a question?

DR. FOSTER: I had one question. And that was, I suppose, the use of quantal versus continuous variables.

When I was reading through the main thing that you sent out, I noticed there were a couple instances where you were saying small, but statistically significant decrease in cholinesterase activity was not considered toxicologically relevant.

I'm not an OP person. So I couldn't tell you what that level is or was.

And I just wondered. If you were trying to compare the utility of benchmark dose from the pup death versus cholinesterase inhibition why you couldn't use a quantal type response. You know, a proportion of pups that had a cholinesterase inhibition of gray (ph) and then whatever you were going to consider to be toxicologically significant.

MR. VILLANUEVA: I think generally that has not been done with any of the OPs we generally model.

DR. FOSTER: It is what was done a lot in the developmental endpoint. You can turn, for example, pup weight into proportion of small pups that was done for the developmental BMD.

I wondered if anyone has considered doing it for other endpoints.

One of the questions you are asking is -- sorry, I'm jumping ahead, is one protective of the other. But you are using kind of different methodologies to get to the same point.

MR. VILLANUEVA: Right. I have never actually -- I have never considered that myself. I haven't heard an OPP that that's the case.

Maybe there are others on the panel that have more to say about that.

DR. PERFETTI: Usually, the difficulty in trying to get, for example, selecting 15 percent cholinesterase inhibition and doing statistics on is your end (ph) gets very small because you have such a wide variation.

You may have -- in a group that you are analyzing 20 rats, if you say 15 percent or 15 to 20

percent inhibition, you may only have six rats and the rest of them are like -- may fall slightly out of it.

Then you say if we keep making it wider, then you are going back to a continuous endpoint anyhow.

DR. ROBERTS: For the record that response was from Dr. Perfetti.

DR. ROBERTS: Dr. Portier.

DR. PORTIER: Just a side comment on that. When you do that, you really reduce the information you have available.

Like you said, your rep goes down to just your number of litters. And if you think about it, if it is culled to four or eight animals, your resolution is to one eight.

So you lose a lot of information in that kind of analysis.

DR. FOSTER: No. I didn't disagree. I think it was more about the endpoint. I think most people agree death is adverse. I'm not quite sure about where this decreasing cholinesterase activity actually becomes an adverse effect.

DR. ROBERTS: Dr. Fischer.

DR. FISCHER: I have the same concern.

Is it -- that is, the biology or the toxicology of selecting a 10 percent decline. So tell me whether there is any biological or toxicological information regarding a 10 percent decline that makes us think that this is a toxic response.

DR. RAFFAELE: I don't really think that there is any consensus. And some of the people on the panel may have more to say about this later on as to what constitutes an adverse, a specific dose which everyone would agree was an adverse effect in terms of brain cholinesterase inhibition.

The 10 percent was based on a lot of the work that was done for the cumulative assessment where they did a large statistical analyses of studies available for a large number of OP pesticides and determined that 10 percent was the statistic limit of detection based on the data available to us.

DR. ROBERTS: Dr. Portier I believe had another question.

DR. PORTIER: You talked about the N logistic model taking into account litter nesting.

You also fit this exponential BMD model. Did that incorporate any kind of inner litter variance component, a nesting component?

MR. VILLANUEVA: No, it didn't. I think generally all the data that we considered it was after the pups started receiving -- well, in the case of where we looked at pups, after the pups started receiving direct dosing. So, no, there was no correlation between the various subjects and the dose groups.

DR. PORTIER: Can I follow up with that?

Why would you expect there is not going to be correlation among litter mates post the lactation period when they get individual dosing?

How does the individual dosing break the litter correlation?

MR. VILLANUEVA: Well, I don't think that it actually does break the litter correlation. But that correlation just is lacking from this model.

And generally, this model was intended to be

used for direct dosing. So that I think was never taken into account, for example, with just adult rats. Of course, that correlation would need not be modeled.

DR. RAFFAELE: I have to go back and check this. But I don't think that more than one pup per litter was evaluated for cholinesterase inhibition models.

DR. ROBERTS: Dr. Heeringa.

DR. HEERINGA: Just two questions.

I would be interested in the nature of the goodness of fit statistics that you used. Is that some grouping type goodness of fit statistics with predicted versus number (ph) of deaths based on the model or is it --

MR. VILLANUEVA: It is the chi squared goodness of fit statistic.

DR. HEERINGA: That's good. Thank you.

And the other issue that -- if we think about lost information, the other piece of that your analysis is giving up is the information on time of death of the pup.

Have you ever looked at the potential of

transforming this N logistic model into a model that incorporates sort of a discrete time logistic survival model that would allow you to estimate survival hazards for these pups under the different regimens?

MR. VILLANUEVA: I'm not sure that's being considered --

DR. HEERINGA: Maybe I should shorten it up. Time is not being brought in -- time of death is not. So you are descritizing (ph) it within these intervals.

MR. VILLANUEVA: Correct. Right.

DR. ROBERTS: Any other questions? Yes. Dr. Reed.

DR. REED: Just a quick question.

There seems to be an issue about, for example, in the cross fostering study that there is an issue about what you include or exclude in the postnatal day one to four counts in terms of pup death.

And I think one of the issues is not to include the pre cross fostering data into that count.

Have you done benchmark dose assessment or modeling for just the pre cross fostering data on pup

death?

MR. VILLANUEVA: No. We did not consider the cross fostering study for any of the benchmark dose analyses.

DR. REED: Could you sort of expand on that in terms of why not looking at that?

DR. RAFFAELE: The main reason that we did the pup mortality modeling only with the DNT study was because, based on the data that we had, that seemed to be the most sensitive study.

The cross fostering study, the lowest dose they used was 3, which was higher than the doses, which the effect was seen in the DNT study.

DR. ROBERTS: Last call for questions on the benchmark dose.

Then let's take a 15 minute break and reconvene. And we will talk about the integration of hazard and dose response analysis. Thank you very much, Dr. Villanueva, for your presentation.

(Thereupon, a brief recess was taken.)

DR. ROBERTS: Our next presentation is by Dr.

Raffaele again. She is going to talk with us about -- make a presentation on integration of hazard and dose response analyses.

DR. RAFFAELE: As you can see, based on the roadmap, this is the last presentation from us for this morning. We're going to briefly go over the various results and try and fit them together for you.

Just to remind you of the issues that we're raising for the panel. The first issue is the relationship between maternal toxicity and pup mortality. And we have sort of talked about two aspects of that. One is the inhibition of brain cholinesterase. And the other is the possible effect of maternal toxicity itself on the pups.

The second issue is the relationship between the duration of exposure and the increase in pup mortality.

And finally, the selection of the critical effect, which would be protective for all populations.

So first let's talk a little bit about the first issue. Specifically, maternal toxicity as a possible cause of pup mortality and briefly compare the results

across the studies.

One of the questions is what data are available to address the issue of the relative contribution of maternal toxicity to the increase in pup mortality.

We have several studies, DNT studies and reproductive toxicity studies. But these studies actually provide very little information which allows us to separate the impact of maternal toxicity on the increase in pup exposure since both the pups and the dams are exposed potentially throughout the treatment period.

In addition, we can't define the relative doses to the maternal animals and the fetus or young pup during lactation because there is no measure of the exposure to the pups.

In addition, as I mentioned previously, the maternal observations are limited and can't really be compared with the pup observations during the DNT study.

However, we did note that there was no indication of excessive maternal toxicity in the DNT study or the repro tox studies based on the types of observations they normally do in those studies.

However, it is likely that there was considerable cholinesterase inhibition, especially at the higher doses.

The cross fostering study was conducted to provide more information regarding the relative contribution of maternal and pup toxicity to the increased pup mortality seen in the studies.

In this study, detailed maternal observations indicated that there was some toxicity in the maternal animals at both 3 and 6 milligram per kilogram dose.

But it is not possible to tell the reason for the maternal behavior, because it has been shown in the past that maternal behavior can change based on the behavior of the pup or the health of the pup.

In addition, the difference in pup mortality was not strictly correlated with the differences in maternal symptoms. There was an increase in restlessness and scattering of the pups at both the 3 and 6 milligram per kilogram dose, but there was not a clear increase in mortality at 3. And only at the higher dose was there a clear increase in pup mortality.

There were no milk measures. So we don't know exactly what the pups were exposed to. Although, the decreased cholinesterase inhibition at PND 4 might imply that there was little exposure to dimethoate in the maternal milk, we don't know what the cause of the maternal mortality was. And it is possible there may have been exposure through the milk to other metabolites of dimethoate, which could have contributed to the mortality.

In addition, there appear to be a relationship of the timing of exposure to the timing of the increased mortality in that those pups which were exposed in utero tended to die earlier during the lactation period than those who were exposed only postnatally.

A second possible contributor to the increased pup mortality would be brain cholinesterase inhibition.

Brain cholinesterase inhibition was seen in all the studies in which pup mortality was increased. But there doesn't appear to be any direct correlation between the magnitude of the inhibition and the magnitude of the increase in mortality.

In the various studies we have described,

mortality occurred at different studies and at various levels of brain cholinesterase inhibition.

In some studies, for example, the range finding study or the cross fostering study, there was a considerable amount of cholinesterase inhibition, for example, at 3 milligram per kilogram dose where no increases in pup mortality were seen.

However, in the main DNT study, increased mortality was seen in pups in levels of exposure that caused relatively low levels of brain cholinesterase inhibition.

This table just shows briefly in the various studies which doses cause brain cholinesterase inhibition in either the pups or adults when that data was available.

And also the dose levels which show the increase in pup mortality.

You can see, I'm not going to go through all this, but you can see if you look at it that there really doesn't appear to be any kind of a direct correlation between the doses causing these two different effects.

Overall, again, to remind you, we saw increase

in pup mortality in lots of different studies. But the doses at which it occurred varied. And the incidence of maternal behavioral changes did not seem to correlate with the increase in pup mortality.

There were no behavioral changes seen in the DNT and related studies. And the changes seen in the cross fostering study didn't seem to be specific to those doses which caused increased mortality.

With respect to cholinesterase inhibition, the magnitude of maternal brain cholinesterase inhibition did not correlate with the level of pup mortality.

As I said, there seemed to be inhibition in some studies with no death and death in studies with very little inhibition.

So, therefore, we don't believe that the cause in mortality can be definitively established based on the data available to us at this time.

The second issue has to do with the exposure duration which may be required before you see an increase in pup mortality.

A default assumption in doing our risk

assessments is the developmental effects may be caused by single exposure during a critical period.

There are a lot of literature data available which support this assumption, that developmental effects can occur as a result of a single exposure.

Standard protocols used by the agency do not provide information which allow us to evaluate the validity of this assumption in particular case since almost all the studies involve continuing exposure to pups or fetuses and dams throughout this study period. And that would hold true for both developmental studies and reproductive toxicity studies.

The cross fostering study conducted by the registrant included groups with isolated either pre or postnatal exposure. I have listed on the slides the various types of exposure which were available.

And since the group sizes were similar to those used in the DNT study, the sensitivity of this study to detect increases in mortality should be similar.

In the cross fostering study, increases in mortality were seen at the 6 milligram per kilogram dose

for all the treatment scenarios, both the isolated pre or postnatal treatment and the combined pre and postnatal treatment.

So at the 6 milligram per kilogram dose, we still don't have information which would allow us to determine whether the mortality was the result of a single exposure or repeated exposure.

However, at 3 milligrams per kilogram, there was no clear increase in mortality following multiple doses to the dam, up to 15 doses pre natally or 11 doses postnatally.

At 3 milligrams per kilogram there was no group that had the combined exposure. So we can't compare that finding with the DNT study.

Based on the lack of increase in pup mortality at doses of 3 milligrams per kilogram, following up to 15 maternal doses, we feel it is reasonable to conclude that these deaths or the increase in mortality at this dose should not be considered to be the result of a single exposure.

But at higher doses, we still don't feel we have

sufficient information to determine that.

The third issue has to do with the relationship between doses at which we see brain cholinesterase inhibition versus doses at which we see increases in pup mortality.

Evaluation of the brain cholinesterase data results in the finding that there is a consistent dose relationship across multiple studies in level of inhibition seen with a given dose of dimethoate.

This seems to hold true in the BMD analyses regardless of the differing durations of exposure in these studies and regardless of whether the administration was occurred as a result of mixing with the diet or administering directly through gavage.

In addition, there was a consistent relationship across the ages that were evaluated with no apparent difference in the dose response curve for the younger or the adult animals.

Based on this consistent -- since we have such a consistent dose response for the cholinesterase data and using the benchmark dose analyses techniques, we felt that

it was possible to compare the doses at which we were likely to see adverse effects on these two different endpoints.

As Philip has discussed, we chose the BMDL 10 for brain cholinesterase inhibition and the BMDL 5 for the increased pup mortality as the appropriate effect levels for comparison.

As you have seen in Philip's presentation, the lowest BMDL 10 for brain cholinesterase inhibition following a repeated dosing was of .2 milligrams per kilogram per day. It was lower than that of .2 7, which was associated with the 5 percent increase in the background rate of pup mortality.

Since we used the main DNT study to do the benchmark dose for pup mortality, we felt this was the most sensitive dose at which we would see the increase in pup mortality.

I just point out again that the BMD levels are different for the two different endpoints. And that was because -- based on our analyses of the doses or the effect levels which would be considered adverse.

A similar comparison can be made for the single exposure. We have calculated the benchmark response. The lowest BMDL 10 for brain cholinesterase inhibition was 1.3 milligrams per kilogram per day.

Again, this is a lower dose than that which we believe causes -- might cause an increase in pup mortality following a single dose, which was only seen at doses greater than 3 milligrams per kilogram per day.

That was based on the results of the cross fostering study.

In conclusion, we feel that exposure to dimethoate at doses which are insufficient to cause increases in brain cholinesterase inhibition would also not cause increases in pup mortality.

Therefore, use of brain cholinesterase as a critical effect would also be protective against adverse effects in offspring.

That concludes this portion of the presentation.

DR. ROBERTS: Thank you, Dr. Raffaele. Let me ask the panel members if they have any questions.

DR. PESSAH: I was wondering why the other

indices that you measured such as the behavioral endpoints aren't included in the study?

DR. RAFFAELE: We didn't really have any issues related to interpreting those results. Although the data are available to you in the DER that we have provided as part of the background information, we didn't have any specific issues with that that we felt we needed additional information on.

DR. ROBERTS: Dr. Chambers.

DR. CHAMBERS: Were the behavioral observations on the mothers, dams -- when they are talking about restlessness and all, was that conducted over a long period of time or just a short period of time?

DR. RAFFAELE: They were conducted during the lactation period. So that was up to postnatal day 11. I believe they were conducted -- four observations per day during that period.

DR. CHAMBERS: Were the four observations per day, did they all show the same sort of observations then? Or did they sort of wane during the course of the day or do you know?

DR. RAFFAELE: I don't know the answer to that question. I'm not sure if the data were presented to us in a way that we could figure that out. But we could try and find out.

DR. CHAMBERS: I have an additional question too. You conclude that the magnitude of maternal brain cholinesterase inhibition did not correlate with the level of pup mortality.

That cholinesterase inhibition would not have been in the same dams that had lost litters, though. Is that correct?

DR. RAFFAELE: That's most likely correct, yes.

DR. CHAMBERS: Different animals.

DR. RAFFAELE: Actually, it was a different study because of the companion cholinesterase study. There was no increase in pup mortality in that study. And cholinesterase was not measured in the main DNT study or the cross fostering study.

DR. ROBERTS: Dr. Cory-slechta.

DR. CORY-SLECHTA: Do we know how terms -- whether there were operational definitions of measures

such as restlessness or scattering? So what defined restlessness? How would that be measured?

And if it was measured repeatedly, what was the reliability of that measure? Was it measured systematically across all the dams?

And the same would be true of scattering. So what operationally defined scattering?

DR. RAFFAELE: I don't remember that there was a specific definition. But I can ask some of the people who actually did the study review who are here who may remember more specifically than I do.

No. They said there was not.

DR. CORY-SLECHTA: No definition. Thank you.

MR. HAZELDON: My name is Keith Hazeldon. I work for the contract lab that actually conducted the studies.

As far as this question is concerned, the dam restlessness and this kind of thing was -- these kind of things are kind of difficult to quantify, difficult to set specific criteria for.

So it was done as consistently as it could be by

technicians who were communicating with one another and trying to cull these things consistently one with another.

DR. CORY-SLECHTA: Can I follow up?

DR. ROBERTS: Sure.

DR. CORY-SLECHTA: So it might be fair to conclude that if you and I were to separately observe those dams, we might come up with different conclusions about whether or not they were restless.

MR. HAZELDON: I suppose you could say that. That would be true, yes, because these things are subjective judgments.

DR. CORY-SLECHTA: I wouldn't necessarily agree with that premise to begin with. But they can be operationally defined. That's why I asked the question.

DR. ROBERTS: Dr. Collins.

DR. COLLINS: Were they done blind or did people know the dose levels when they made these observations?

MR. HAZELDON: Yes, they were observed in blind.

DR. RAFFAELE: I believe in the study report it says they were not done blind. We can go back and check.

MR. HAZELDON: Well, the dose levels were not

identified.

DR. CORY-SLECHTA: When the measurements were done, if they were done by the same individuals, do you have any idea or different individuals about the reliability across what the interobserver reliability of those measures was?

MR. HAZELDON: Yeah, well, we were quite careful about that. So that the technicians who were involved in these studies were -- as I said, did communicate with one another over these kinds of issues, because we are aware that there is a subjective element here and at least to try and keep that element consistent between the different observers.

And there was actually a very minimal number of observers involved anyway, perhaps two or three people altogether to do those specific observations.

DR. RAFFAELE: We did specifically ask the question regarding whether the observations were done blind during the process of reviewing this study.

And the information that we received back from the registrant at the time indicated that they were not

done blind.

Because of the complication of the study, that they said that it would have been very difficult to do that without potentially causing additional errors in the data.

MR. HAZELDON: Yes. For the cross fostering study, that's true.

DR. RAFFAELE: Right. That's the one we were talking about.

DR. ROBERTS: Thank you. Dr. Chambers and then Dr. Pessah.

DR. CHAMBERS: Let me reask the same question I asked Dr. Raffaele a few minutes ago. Were the observations of restlessness and all consistent through the four daily samples? Or did they wane with time during the day after dosing?

MR. HAZELDON: They were -- I can't answer that directly. All I can say is that there were -- that the observations were spread fairly evenly across the day. And the same criteria we used across the days.

But what I can't tell you is whether the

intensity of those things waned across the day or not. We don't have that information.

DR. CHAMBERS: So were some of the observations shortly after dosing then?

MR. HAZELDON: Yes.

DR. ROBERTS: Dr. Pessah.

DR. PESSAH: Sorry to hammer this point, but I think it is an important one. There was no effort to do a cross check on different observers for consistency and scoring restlessness and scattering?

MR. HAZELDON: Well, that was done while the study was in progress so that people were able to compare notes as they observed the animals effectively. There was no formal test of it.

DR. ROBERTS: Dr. Harry.

DR. HARRY: This would be the appropriate time to ask questions while we have him sitting in the corner here? Or would you rather us wait until --

DR. ROBERTS: There will be a presentation in the public comment period, and I think that's probably the best time to maybe ask other questions about the

registrant study.

Yes, Dr. Foster.

DR. FOSTER: I suppose one of the things I was grappling with is this pup mortality, something seen as class effect. You must have looked at a lot of DNT studies.

I think we're trying to grapple with how much of this is due to its pharmacological activity and how much of it isn't.

DR. RAFFAELE: It is not something that's seen for all the organophosphate pesticides.

I haven't looked at the data for all the OPs. I believe that I recall that there were some others, OPs, that showed this effect in the repro study, but it was generally at higher doses compared to the cholinesterase inhibition than what we see with dimethoate.

So usually it was mainly high dose effect, if I'm remembering correctly.

DR. ROBERTS: Any other questions for Dr. Raffaele, Dr. Villanueva, Dr. Locke, anyone from the agency on their presentations?

Hearing none, I would like to thank the agency scientists for their very informative presentation and their patience in answering our questions.

I think it has been very helpful in terms of understanding the agency's analysis and interpretation, bases for the decisions that were made. Thank you very much.

I would like to proceed now to the next phase of the agenda, which is public comments.

We have as a first set of individuals that requested the opportunity to address the panel representatives from Cheminova.

There are a number in the group. I'm not sure what the order of the presenters will be or the scheme, but I would like to ask them to approach at this point and request that all of the individuals who are going to address the panel identify themselves, please.

MS. ALLENMAG: Good morning. My name is Diane Allenmag. I'm director of regulatory affairs for Cheminova. I want to thank the panel and the agency for giving us this opportunity to speak today.

Earlier in the year when EPA scheduled the SAP meeting, Cheminova decided to ask a number of independent experts to review our data. And as a result, the presentation today will be by these experts rather than Cheminova.

And, therefore, while I will remain here, that's all I have to say. I'm going to turn it over to Dr. Abby Li.

DR. LI: I'm going to introduce the three presentations that you will hear today. We are going to discuss the key issues for the risk assessment for dimethoate.

As Dr. Kathleen Raffaele discussed earlier, there are two critical effects that were defined for the DNT study. There is pup mortality and brain cholinesterase inhibition.

Our ultimate goal was -- in evaluating this data carefully, is to select a point of departure that will be protective of all exposed populations of concern, including the fetus, infants and children.

And in order to accomplish that, we felt it was

really important to go back to the original data -- next slide -- to go back to the original data.

All the dimethoate data that we felt was relevant for fetus, infants and children. So we evaluate much of the data that Kathleen discussed this morning.

Not only did we look at the reports for the developmental neuro tox and the related oral gavage studies, but we looked at the reports for the multi generation rat reproduction studies, the teratology studies and the benchmark dose analysis that was conducted by EPA and then independently by Dr. Reiss and Gaylor.

So the presentations that we are giving today are based on two expert papers that I believe the EPA has made available to the SAP.

The first is entitled the BMD met analysis of critical effects. This is authored by Dr. Richard Reiss and David Gaylor. And both Rick and Gaylor have substantial experience using statistical models for risk assessment.

And David in particular has been a pioneer in the field of benchmark dose application to cancer and non

cancer endpoints.

And, in fact, he was one of the expert consultants that helped develop the EPA guidance on the benchmark dose.

The second paper is entitled dimethoate, key issues for the assessment of potential human health risks. And the authors are Dr. John DeSesso who is from Mitretek. He is a developmental biologist and toxicologist who is an adjunct professor at several medical schools teaching embryology and developmental biology and toxicology.

And among his many accomplishments was the past president of the teratology society.

Dr. Carl Keen is the chair of the department of nutrition at UC Davis. And he has spent much of his research career looking at mechanisms by which maternal toxicity can cause developmental toxicology prenatally and postnatally.

Dr. Rebecca Watson is a molecular toxicologist who is with Mitretek.

Dr. Laurie Haws is a toxicologist at Exponent.

She has research experience in cross fostering studies and developmental toxicology. She just joined Exponent.

Earlier she was at the equivalent of -- Texas EPA as the manager of the toxicology and risk assessment department.

And my name is Abby Li. I'm a neurotoxicologist with experience and expertise in risk assessment.

I have conducted and monitored many adult and developmental toxicology studies. And we aren't as good as EPA. We required a lot more help than they had.

There is phenomenal amount of data. I just want to acknowledge the efforts of the EPA in really looking carefully at the data.

We had the help of Dr. Joe Ross, who carefully evaluated the neuro behavioral data. Dr. Rudy Richardson, who, unfortunately, because of recent surgery is not able to be here with us, but he was extremely helpful in helping us to understand the cholinesterase data.

Mr. Keith Hazeldon, who you just heard from, is from Huntington Laboratories. He was critical to our keeping all of the individual pup and that dam data

straight.

It was a phenomenal amount of information. And he also provided some information on study conduct in terms of personnel and dosing that we could not easily get from the report.

I think there was a little bit of confusion. He thought that you were talking just about the developmental neuro tox studies. There are maternal observations done to the dams which are conducted blind using an explicit scale that were done on the DNT studies. And that was conducted blind.

So we also had the benefit of Dr. Don Oshannassy's (ph) expertise, who was the study monitor for the four studies. He helped us understand the rationale for the study designs based on the agreements and requirements of EPA.

What we would like to do in the next two hours and hopefully less is to share with you our approach to analyzing the data, which led us to the following conclusions.

First, as discussed earlier, we agree that brain

cholinesterase inhibition and pup mortality are critical endpoints of concern from the gavage studies. We believe that the interpretation of pup mortality is confounded by a strong maternal influence on them, pup mortality.

We agree that adults are more sensitive than offspring to effects of dimethoate on brain cholinesterase inhibition.

And taken together, we agree with the EPA that selection of the adult brain cholinesterase inhibition as a point of departure is protective of pup mortality.

And we took sort of two different but very closely related approaches to evaluating the data. We looked at the data from a quantitative dose response analyses approach. And then we also evaluated the data from a more qualitative biologically based analysis.

And we would like to share those two approaches with you today. The first speaker will be Dr. Richard Reiss and David Gaylor who will discuss their statistical evaluation to determine the most appropriate endpoint for dimethoate risk assessment.

Drs. John DeSesso, Carl Keen, Rebecca Watson and

Laurie Haws will discuss the maternal influence as a contributor to pre and postnatal offspring wellbeing and as a driver for the risk assessment for dimethoate.

I will briefly discuss the weight of evidence evaluation of other relevant studies, and then wrap up with highlights of the key issues that we think are most important from these two presentations that need to be considered in the risk assessment for dimethoate.

So Rick.

DR. ROBERTS: Dr. Li, let me suggest. Since you have a series of presentations, that perhaps we pause after each presentation to give the panel the opportunity to ask questions.

DR. LI: That would be great.

DR. REISS: Well, I'm really pleased to be here speaking in front of the SAP. Some of you may remember I was here a couple months ago where I think I spoke for two and a half hours and then answered questions for a day and a half. This will be a considerably shorter presentation and hopefully a somewhat shorter question and answer period afterwards.

I would like to acknowledge my coauthor, Dr. David Gaylor. As Abby said, Dr. Gaylor is one of the most cited authors in the field of benchmark dose modeling. So we're really pleased that he could participate in this project.

As you know, EPA just presented a benchmark dose analysis that came to very similar conclusions as our analysis did.

We submitted this originally back in just before or after the last SAP, scheduled SAP meeting. And EPA went ahead and did an analysis that was pretty similar but different in some ways than our analysis, although we came to the same general conclusions.

Let's just first start with what we agree with. First, we agree with EPA that benchmark dose modeling is a very appropriate tool to compare the effect levels of different endpoints.

We also agree with EPA about the choice of BMD models that were used for modeling pup mortality and cholinesterase inhibition. And the various points of departure, the BMD 10 for cholinesterase and the BMD 5 for

pup mortality that EPA chose.

We're not saying that either of these BMD 10 for cholinesterase or BMD 5 for pup mortality is the right level in terms of an effect level. These are the levels that are used for risk assessment.

So the question we're trying to answer is if we use a BMD 10 for cholinesterase inhibition, will that be protective of the commensurate level for pup mortality.

We also agree with EPA that using brain cholinesterase for risk assessment is protective for pup mortality, meaning the -- that's the ultimate conclusion of the assessment.

So to complement that analysis done by EPA, we conducted a meta analysis of multiple studies to provide a more statistically robust analysis.

We have a unique situation here in that Cheminova conducted four very similar studies for measuring cholinesterase and pup mortality over about the same time period at the same laboratory.

So when I say a meta analysis, some people may think of studies which take various epidemiologic studies

and try to combine them together to get some sort of joint result.

This is a little bit different in the sense that we have studies done with identical protocols relative to the endpoints, we're talking about. They were done about the same time, in the same laboratory.

So really, what we have here is replicate data. So we think -- we're on very strong grounds in doing a meta analysis with this data set.

As you will see, the results strengthen the conclusion that the use of brain cholinesterase is protective of pup mortality.

We considered some other issues using BMD modeling that we think are answerable with that technique in this presentation.

First, is the relative comparison between pup mortality and cholinesterase inhibition the same for the dietary route?

As you might expect, the dietary route would be a more relevant route of exposure for many of the risk assessment applications you would have for dimethoate

whereas the DNT, cross fostering, et al., studies were done by the gavage route.

As you will see, there is a significant difference when you look at the different routes of exposure.

We also look at what is the most sensitive route of exposure for cholinesterase inhibition. We have studies with gavage, dietary and dermal routes. And we will compare BND as a cross for those different routes of exposure.

To provide a guide to the presentation, I'll just sort of go along and tell you what we're finding. I have put a series of five questions that we'll answer as we go through the presentation.

First, what is the most sensitive endpoint, pup mortality or cholinesterase inhibition for the gavage studies that we have been talking about?

What is the most sensitive subpopulation for gavage in terms of cholinesterase inhibition, brain cholinesterase inhibition?

And then we'll look at what is the most

sensitive endpoint for the dietary studies, whether it is cholinesterase or pup mortality.

We'll also look at what is the most sensitive route of exposure, dermal, gavage or dietary for brain cholinesterase inhibition.

And then we'll finally look at what is the most sensitive route of exposure overall for pup mortality, whether it is dietary or gavage.

Let's talk about what sort of data we have first for gavage. We have four studies. As I said, they were conducted at the same laboratory with similar designs for the critical endpoints, cholinesterase inhibition and pup mortality and about the same time, within several years of one another and all relatively recently.

First, the DNT study, the main DNT study measured pup mortality. It didn't include brain cholinesterase because that was measured in a companion cholinesterase study that was done at the same time.

And that companion cholinesterase study also included some measurements of pup mortality.

On the range finding study for the DNT, included

both pup mortality and brain cholinesterase inhibition.

And finally, the cross fostering study included two dose levels that weren't cross fostered, the control group and the 6 milligram per kilogram dose group.

So doing the meta analysis, we didn't want to include any of the groups that were cross fostered. That would be difficult to interpret it. It would be difficult to figure out how to include that.

But we did have two dose levels in that study that were cross fostered, so we felt that we could add those into the meta analysis.

So what do we end up with? This shows a graph by dose group, by 6 dose groups, the number of litters for our meta analysis with all the four gavage studies combined and with the DNT study only.

With the DNT study only, you see we had 96 litters and four dose groups. When we combine all of the gavage studies together, we get more than double that, 220 litters and two additional dose groups.

So for benchmark dose modeling, this is very helpful. We have two additional dose groups, more than

twice as many litters. We can get a more statistically robust answer.

So this is the pup mortality results for the DNT study only. So I'll get to the meta analysis in a moment. And this is the graph that comes out of EPA's benchmark dose modeling software showing the model fit.

This shows you the fraction affected meaning the fraction of pups in a litter that were affected. So this is using nesting within the litter and by the dose level.

And you see a relatively good fit of the data. And it gives a BMD of .52 and a BMDL of .30, which was almost identical to the result that EPA got when they modeled the same data.

The goodness of fit here is .21. That's evidence of an adequate goodness of fit. You sometimes might see -- you might be confused. You might see -- you are always looking for a P value less than .05.

In this case, what we're doing is we're doing a goodness of fit test of this curve versus these data points. And if it is a good model, we don't want to find statistical significance.

If the model we developed wasn't adequately fitting the data that are represented there, then we would get a significant P value and we would conclude that the model was significantly different than the data and we would reject it.

What happens when we add all of the gavage studies together. We got a BMD of 1.5 and a BMDL .96, much higher than we have when we just looked at the DNT study only.

However, we got a relatively poor goodness of fit with a P value of .03. You can see the reason for that is here. We have this 6 milligram per kilogram dose group getting about the same mortality as the 3 milligram per kilogram dose group and the model's just not prepared to model that sort of sloping off effect if it is real in these data.

What a common technique used in BMD modeling is to drop the high dose group. This is something that is recommended in EPA's guidance documents.

And the reason is you are mostly interested in what is happening down at the low dose group. You want

your model fit to be most influenced by the data at the low dose group.

And also, biologically, there could be something happening at that high dose group that's not explained by the model. Let's look at the next slide.

This is when we drop the high dose group and we get a revised fit. And the goodness of fit went from .03 to .49 by dropping that high dose group.

We still have twice as many litters as the DNT. I think there is 188 litters even after we drop the high dose group compared to the 96 in the DNT study.

We ended up with a BMDL 5, the lower limit of .64 milligrams per kilogram which is about twice as high as the result you get with the DNT study only.

We're not advocating taking out any data, but as an intellectual exercise and something that I think illustrates, the large impact that a single total litter loss had in the .5 milligram per kilogram dose group in the DNT study, in that litter there were -- seventeen out of seventeen of the pups died in the first four days.

And although total litter losses they do occur

in controlled groups sometimes, although they are relatively rare, we did observe this one total litter loss in the .5 milligram per kilogram dose group and it has an enormous impact on the results.

I took out that one total litter loss in the .5 milligrams per kilogram dose group, which is just removing one of the 24 litters at that .5 milligram per kilogram dose in the DNT study. That's only one out of a 188 of the litters that made up the entire meta analysis. So just one out of 188 of the litters in the meta analysis.

In that case, you get an excellent fit where the model really predicts well the 3 milligram per kilogram dose, and the .5 milligram per kilogram dose you get a goodness of fit, which is better than any of the other models at .7. And you end up with a BMDL of 1.

That compares to .64 when I included this dose group. So taking out this one, I'm sorry, one litter increased the BMD by 55 percent. And the BMD out here at one is about three fold higher than what you would get with just the DNT study only.

We're not advocating taking the litter out. We

don't want to throw out any data, but this is just an interesting exercise to see what the impact of that single total litter loss had on the overall results.

So what did we end up with? With the DNT study only, we got a BMDL of .3. And the best model we found is when we use all the gavage data without the 6 milligram per kilogram dose, and in that case we get a BMD of .64.

When we included the high dose, we got a relatively poor fit, but a higher BMDL. So I think we made the conservative choice in taking out the high dose and we end up with this value of .64. That's the value we're going to carry forward to do our comparisons.

I want to make some brief comments about the appropriate statistical units in reproduction studies. There are some comments in the documentation provided by EPA regarding whether the litter or the pup is the appropriate statistical unit.

Specifically, EPA says following weaning the pup is the appropriate choice. But between birth -- they said between birth and lactation, I think they mean, birth and weaning the choice is more complex.

Following weaning, this may be a more controversial issue, but fortunately we don't need to grapple with that to understand the dimethoate data because most of the pup deaths occur within the first four days.

However, between birth and weaning, we believe the choice is not really more complex. We think the statistical literature, EPA guidance, if you look at their developmental toxicity guidance, and the scientific literature all support the litter as the only appropriate statistical unit during the period where pup mortality was observed in the dimethoate DNT, like between days 1 to 4 at the very least.

The report we provided includes some citations to this guidance, some ILSI guidance and some scientific literature citations that back up that point.

From a scientific perspective, what we are saying is say when you have that total litter loss in the .5 milligram per kilogram dose group, you had 17 pups that died all within a few days of birth, if you don't include the litter as the statistical unit you will be including

all those seventeen of those deaths as independent events.

You would include each one of them as a degree of freedom in your statistical analysis. That's flawed because something was clearly happening in that litter with the dam. Those seventeen pups didn't all die independently. They all died as a result of some other, you know, action that occurred with the dam.

Now, for the BMD modeling, I think we completely agree with EPA. We use the same dose response models. And all of these models are based on what is called litter proportions.

You take each litter. And if there were five deaths and 10 pups in that litter you would assign that an incidence of .5 or a litter proportion of .5.

So that in this way it treats the litter as the statistical unit as the end. Essentially, the degrees of freedom, but it accounts for each pup death by considering the magnitude of pup death in each litter.

So a litter that had one out of 20 deaths wouldn't be counted the same as one that had 10 of 20 deaths. You would be able to account for that magnitude

of pup death in the model. And the BMD model does that. It is a nested model that accounts for all of the pup deaths while using the litter as the statistical unit.

Let's turn to the cholinesterase data and some of the other exposure routes. As we just talked about, we have four gavage studies related to the DNT. We also have, for dietary, we have a range finder, one GEN. We have two two generation reproduction studies, one in 1992 and one in 2003. And we also have a 28 day study.

And then fortunately, for dermal exposure, we also have a 28 day study. We have an ideal way how to compare dietary and dermal exposure.

I'm not going to go through all of the cholinesterase BMD values that we found or the model in a lot of detail. It is essentially the same model that EPA used, but just to give you an example fit which shows how well the model worked.

This shows the adult female data on day 11 for the DNT study. And you see the green points here are the actual observed cholinesterase levels of the study.

The red line right here is the BMD model fit.

You see the model fit -- the exponential declining model really fits those data quite nicely.

The dose spacing in the DNT study was very good in that since we're looking for BMD 10, there was almost always a dose or at least in many of the subpopulations there was a dose right around 10 percent cholinesterase inhibition.

So that really reduces the uncertainty in trying to estimate a 10 percent level. You see -- you get a good fit and your BMD level is right around one of the dose points.

So let's look at some of the results that we got for the BMDLs for pup mortality and cholinesterase.

Again, I'm going to start by just looking at the DNT study and then we'll turn to our meta analysis in a moment.

For pup mortality, we got a value of .3 for postnatal day 1 to 4. For 5 to 11 or 4 to 11, the value was higher. So we didn't include it on the chart.

So if you are comparing that with the cholinesterase levels, let's look down and see what they

look like. The GD 20 -- the dams had about the same BMDL for cholinesterase. The adult females and adult males also had about the same level, all at about .3.

If you look at the postnatal day 11 males, which had one direct dose, they had much higher BMDLs, 1.5 -- 1.2 and 1.5. Probably because they are not exposed as much.

The PND 21 males, they were exposed 11 consecutive times. And they had BMDs of around .5 and .48, which is interesting. Still higher than the dams and the adult males a and females. And the GD 20 fetuses also had a BMD of .73, which is higher than the adults.

So what you see here is that the adults are the most sensitive for cholinesterase inhibition. And the value that you get for cholinesterase BMDL compares quite well with the value you get for the BMD for pup mortality.

And this is basically the data -- similar to the data that EPA showed you. And they get about the same answer.

If we look at the BMDLs for the pup mortality and the cholinesterase when we combine the gavage studies,

we get a very different result.

For days 1 to 4, for pup mortality, as I showed, you we got a BMD of .64. Adults still stay about the same because there was no additional data for adult males at day 11. So they were .3 6 and .3 2.

There was some additional data for the dams in the range finding study. That went down to about .2 when you added that additional data in there.

So what you see here is you have your pup mortality BMDL is about 2 to 3 fold higher than the dams or the adult males and females. So in this case, cholinesterase is the most sensitive endpoint.

This slide is very similar to the last one, but instead of comparing the lower limit of the BMDs, we compared the central estimate of the BMDs.

So you see the BMD 5 for pup mortality was 1.1 milligrams per kilogram and the BMD for cholinesterase was anywhere from .36 to .47 for males and .29 for dams. So you get a very similar conclusion if you look at the lower limits.

The cholinesterase is about 2 to 3 fold lower

BMDLs than the pup mortality.

This is a better graph, I think, for the statisticians, puts everything on the same scale with both -- whereas these lines show the midpoint or the central estimate of the BMDs and then the uncertainty bounds show the uncertainty and the lower bound shows the BMDL.

So we're interested in knowing what the most sensitive levels for cholinesterase inhibition might be used for risk assessment.

These are adult males, adult females. And the dams, they are down here. Then you see the pup mortality range up here. And those are statistically different.

So there is a statistical difference between pup mortality and cholinesterase. So you can conclude that cholinesterase is a more sensitive endpoint in this regard.

Let's answer the first two questions. What is the most sensitive endpoint for gavage. It is brain cholinesterase. And it is about 2 to 3 fold more sensitive than pup mortality.

What is the most sensitive subpopulation for

gavage. It turns out to be adult animals. It is less sensitive than pups or fetuses.

I'm going to answer the rest of the questions more fast. Let's look now at comparing cholinesterase and pup mortality from some of the dietary studies.

The dietary route may be more relevant to risk assessment. And let's see if we get the same result as we do when we compare the gavage studies.

Now, I think as EPA discussed, there was no clear effect to pup mortality in either of the two generation reproduction studies. So what I did is I culled the BMDL greater than the highest dose, which in this case was 6 milligrams per kilogram.

Then we calculated the BMDL 10s for cholinesterase inhibition. And then around .5 for the females and the males. I didn't put all the generations on, but the results are all relatively similar.

So in this case you could say the cholinesterase inhibition was about 12 fold more sensitive than pup mortality for the 1992 dietary study.

For the 2003 dietary study, a very similar

result. Again, no clear effect of pup mortality. So I culled the BMD greater than the highest dose of 6.5 milligrams per kilogram. And the cholinesterase levels were about .7 and .5 -- .6 for the females and males.

And in this case it's about 10 fold more sensitive. Cholinesterase is about 10 fold more sensitive than pup mortality.

What is the most sensitive endpoint for dietary studies. It's brain cholinesterase by a wide margin. Greater than 10 fold more sensitive than pup mortality.

We can also compare across exposure routes. We have studies, a 28 day study for the dermal route, a 28 study for the dietary route of exposure. And we have at least 15 days of exposure, consecutive days of exposure, 11 to 15 days from the DNT studies.

There are for the dietary two generation studies. There are some longer exposures to cholinesterase inhibition which gives some lower BMDLs than what we're showing from the 28 day study. But we thought the best comparison would be using similar durations of exposure.

So not surprisingly, the dermal comes out much, much higher at 25.8. That was the lowest BMDL from that study. The dietary came out at .7 and gavage came out at .19.

So I think a rather intuitive result, the dermal greater than the dietary, greater than the gavage. Actually, if you put it -- that's in terms of BMDLs. If I put it in terms of sensitivity, I would say the gavage is greater than the dietary, greater than the dermal.

We can also compare for dietary and gavage. We don't have a dermal pup mortality study, but we have -- for dietary and gavage, we have the 2 gen, repro studies, which, again, showed no consistent levels of pup mortality.

So we culled those greater than 6 and greater than 6.5. If we look at the gavage studies, when we looked at the DNT study only, we got a BMD of .3. When we did our meta analysis, which we think is a more statistically robust answer, we got .64.

You can see there is a very wide difference where clearly across the dietary route the pups were less

sensitive to pup mortality.

So the most sensitive route of exposure for pup mortality, it is gavage and then dietary.

In summary, this whole presentation was mostly geared toward answering question 1.3, which was, please comment on the evidence that supports or refutes the statement that brain cholinesterase inhibition can be used as the endpoint for dimethoate risk assessment for all durations of exposure.

We believe this statement is fully supported by the EPA BMD analysis that was presented a while ago. And our meta analysis we think is more statistically robust. And it strengthens the conclusion by adding more statistical power and finding a larger difference, 2 to 3 fold instead of about equal between cholinesterase and pup mortality.

This conclusion is further supported by the dietary BMD analysis, which is probably more relevant for risk assessment where the gap between cholinesterase inhibition and pup mortality was greater than 10 fold.

That concludes the presentation. And David and

I would be happy to take any questions you might have.

DR. ROBERTS: I think Dr. Riviere has the first one for you.

DR. RIVIERE: I have one question on the goodness of fit statistics. Exactly what is this statistic, what is its range and when is it considered bad?

DR. GAYLORD: I'm David Gaylor. I'm glad to be here. I have worked with a number of you in the past. And I applaud the benchmark dose analyses that were conducted by the EPA. They did a fine job.

The question was -- Rick discussed that earlier. The goodness of fit is a chi squared test. And the P values range from 0 to 1.

If you get a P value less than .05, that's kind of the typical cutoff, it means we would reject the hypothesis, reject the model that we use to fit the data.

So a low value of P less than .05 would indicate a poor fit. Typically, we like to get P values greater than .1. Some of them got up quite high, .7. That's almost getting a perfect fit.

I would point out here in the gavage -- I'm sorry, in the dietary study where you are fitting the cholinesterase model, the negative exponential model, that model has 3 parameters in it.

And basically, only have 3 doses, because the controls and the low dose gave almost identical results. So if you fit that full negative exponential model, it is going to curve around and go through the data points. It has to and you have to get a good fit.

So in the fitting I did, I chose not to try and fit that asintotic -- I started out doing that. And the asintotic values were at what you would predict at high doses when we don't have high doses in extrapolation.

I would even come up with negative cholinesterase. And not being a biologist, I knew that that wasn't right. So I made the conservative assumption that dimethoate would drive cholinesterase to 0 at high doses.

Maybe that's too stringent. I don't know. But it avoid fitting. I only fit 2 parameters to basically 3 doses. So I had a little better measure goodness of fit.

But that's kind of a long answer to a short question.

DR. ROBERTS: Did you want to follow up?

DR. RIVIERE: Has anyone done like Akaike information criteria or something that would allow you not to fit 3 doses to a curve like that? I mean similar objective value that's saying that there is not enough data here to do a BMD analysis.

DR. REISS: For the pup mortality --

DR. RIVIERE: That's the concern. The cholinesterase, everything obviously --

DR. REISS: Yes, the AIC is the criteria that you use to choose the best fit model for the pup mortality using some various -- they have some various options in the model about intra litter correlations or whether the litter size has an impact on pup mortality.

And all of those choices had very small differences in the results you got. But the criteria is you pick the model that has the best AIC.

DR. ROBERTS: Dr. Cory-Slechta.

DR. CORY-SLECHTA: I'm not a statistician. I

just have one question. You may have talked about it. Does this meta analysis assume that all of the studies that you include have the same power or sensitivity to detect an effect?

And is that a legitimate --

DR. REISS: It doesn't need to make any assumption. It treats all of the data collected in the studies as replicate data. So it doesn't take the result from one study, the BMD result from one study and the BMD result from another study and combine them.

It actually goes into the original data set and takes all of the data out and considers them all as replicate data.

DR. CORY-SLECHTA: But doesn't that assume that each of those data points has the same validity or sensitivity or power?

DR. REISS: When I say a data point, I mean an individual litter. So it is just taking all of the individual litters and combining them together. So it is assuming that each litter is a replicate data point and that it can be combined together.

DR. ROBERTS: Dr. Pessah, then Dr. MacDonald.

DR. PESSAH: I was just wondering. I think I understand the BMD approach. But in reality, the biology says that the pups are -- at gestational day 20, the pups have about 7 fold lower density of cholinesterase than the dams.

And that becomes 4 fold less at PN day 4. Is there any way that the model accounts for that?

DR. REISS: The model calculates the BMD separately for these different subpopulations. So it doesn't combine together subpopulations. The assumption is there is something -- there could be something biologically different in pups at -- well, fetuses at GD 20 or pups at BMD 4.

So the model -- we don't essentially model them together. We model them separately.

DR. ROBERTS: Dr. MacDonald.

DR. MACDONALD: I didn't like what I have been hearing about the interpretation of the P values and the goodness of fit test. Because if you really do have the right model, you don't want all the P values to be large.

You want 5 percent of them to be less than 5 percent. You want 10 percent of them to be less than 10 percent and so on. You are looking for a uniform distribution of all your P values, that is if you are doing essentially a meta analysis over all your model fitting.

DR. GAYLORD: That's basically what happened here. We had some P values all the way up to .99 and some at .01. So we had a whole range. I didn't really look to see if it was uniform --

DR. MACDONALD: I did have a look at the one on -- some of the tables we saw earlier this morning. Had 27 P values. In fact, there weren't enough in the middle. There are some nice big ones and some nice small ones, but not enough in middle to be uniform. It's a bit odd.

DR. REISS: Is that the EPA analysis you are referring to?

DR. MACDONALD: I believe so.

DR. GAYLORD: As I was saying, I think --

DR. MACDONALD: Yes. On page eight.

DR. GAYLORD: There is probably some --

DR. MACDONALD: CHEI model results, sorry, page 15 of 21 of the EPA presentation.

MR. VILLANUEVA: Slide 57 of the EPA presentation.

DR. REISS: If you have our report, I don't know if you have it in front of you, on page 33, we present the goodness of fits values for the cholinesterase. And there are values ranging -- it looks like uniform distribution to me. There are values ranging from, as Dave said, .03, .27. So they range the gamut.

DR. MACDONALD: The other point of course is that if you have got a really weak data, then you are going to get an extremely good fit whether you want it or not. That was well addressed by Dr. Gaylor.

DR. ROBERTS: Dr. Reed.

DR. REED: This is sort of a separate question from meta analysis. I was just curious looking at, which report is this, November the 1st, 2004, response from Cheminova to EPA's daily evaluation record for the dimethoate cross fostering study.

You have actually looking at pup death on day 1,

which is in page 24, table 9. Could you sort of go over that a little bit in terms of whether this kind of data is fit for benchmark dose analysis.

DR. REISS: Sure. I believe that data -- when we do a benchmark dose for pup mortality, the goal is to calculate a BMD 5. The dose level that would cause a 5 percent incidence in pup death.

I believe with that data the incidence is much, much lower than 5 percent on postnatal day 1. So you really couldn't fit that type of model to that data.

Furthermore, for the cross fostering there is only three dose groups. As we said, there is some hazards to fitting a model with only three dose groups.

DR. REED: I guess I was thinking of this as an example data set meaning that with other, the DNT study and the range finding if there is some data available for doing analysis on day 1, that would get you away from the maternal behavior and all that kind of thing.

DR. REISS: That's an interesting suggestion. It is not something we have looked at.

DR. ROBERTS: Dr. Harry.

DR. HARRY: Just a point of clarification. When you were looking, comparing the most sensitive routes, you were looking at pup mortality only from day 1 to day 11?

DR. REISS: For the gavage studies, we broke it into postnatal day 1 to 4 and postnatal day 4 to 11 because there was a cull on -- except in the cross fostering study, there was a cull. The most sensitive period was postnatal day 1 to 4. So in my presentation, that's all that I presented.

In the report, we did calculate BMD values for both periods. When we did the meta analysis, we didn't include the cross fostering study for the second period from PND 4 to 11 because they didn't have a cull. So it prevented us from assuming that they were replicate data.

DR. HARRY: Is there any data at all that says that basically once the animals get to 11 days of age that there is no mortality that you see ever after that?

DR. REISS: I can't answer that definitively. But there is -- certainly the predominant level of mortality in this study was from postnatal day 1 to 4. I don't believe anything beyond that was statistically

significant.

DR. ROBERTS: Any other questions.

If not, Dr. Gaylor, Dr. Reiss, thank you very much for your presentation. Appreciate it.

Dr. Li, did you want to introduce the next speaker?

DR. LI: Yes. Did you want to go ahead with that? It may take at least 30 minutes, possibly more.

DR. ROBERTS: That's fine.

DR. LI: Didn't want to cut into your lunch.

DR. DESESSO: I'm John DeSesso. And I'm going to talk to you on behalf of a whole lot of very talented people about what we saw when we looked at the biology data.

I might point out to you, and I know you all received a large volume of material to look at as we did, we all got involved in this I guess in early July.

So it was probably about the same thing you got, which was this massive amount of information and how you are going to make sense out of it. That's sort of what we were doing. My kids have these things called magic eye

pictures. Have you seen those? Where you stare at them until you go nuts, and then all of a sudden a pattern emerges.

I think that's sort of the process we went through with this. We looked at all these things. We see pup deaths and we see all of these different things. The question was what is in here that makes sense.

And this is the story I'm going to tell you, what made sense to us. The first thing we did in telling you this story now is we are going to discuss what is the relationship of dam to her offspring.

I know this might sound somewhat pedantic. I don't mean it to be that way. I want to just make sure we are all on the same page. What is the relationship?

Obviously, when she is pregnant, there is a very intimate relationship. This is a diagram that shows a rodent uterus. There is nine implantation sites. The vagina is down here. Each of these -- of course, this is a little embryo.

The darkened area would be where the placenta is located. It is inherently obvious that everything that

goes into the baby goes by way of this -- if not by way of the placenta, then by way of some kind of diffusion through the wall of the uterus.

But it is all coming from the mother. So our nutrients are coming from there. The embryo shown here and there are surrounded by fluid compartments. In this case, it is the yolk sac that surrounds that and there is a fluid inside. Obviously, the source of the fluid came from the mother.

The mother has a very big role to play here. We were thinking then -- obviously, she provides the physical environment and protection from her belly and from the fluids.

Homeostatic mechanisms involving the fluid pressure inside the embryo to allow the different organs like the limbs to develop and so on is certainly under her control.

The temperature is obviously under her control. If she gets raised body temperatures, there will be birth defects. If she gets too low body temperatures, the pups will die.

She is the means by way of the placenta for elimination of metabolic waste and she also has control of the respiratory gasses and so on, because that is also happens in the placenta.

And of course she is the source of nutrients, electrolytes, vitamins, so on and so forth. So that is pretty much set. Not much to argue with there.

Let's see what happens after birth. So the question is what happens at birth. Rats are pretty good moms. So let's see the next slide.

Here is a rat. This happens to be a hairless rat with her litter. This is probably at about day 8 or 9. Obviously, the pups are there feeding. In fact, most people -- well, she is the sole source of nutrition for her pups at least until about day 11 or 12. Some authors claim as late as day 15.

But obviously, the calories are coming by way of the mom. So let's see the next slide.

If we are to draw up a chart, now this is a hypothetical chart, to try to understand what is the relationship of what mother supplies to baby over the

course of the time when the baby is an embryo until the time when the baby starts to get out on its own, that's what is on this chart.

So we look at the embryo. We look at the neonate from days 0 to 4. We look at the pup as it develops from 5 to 12. And then from 12 to 21. And then after 21 we will call that the juvenile period when the weaning has taken place.

And if we look at the same things we talked about before, we talked about respiratory gasses, waste elimination, physical protection, thermal homeostasis and nutrient supply, and just qualitatively say how much of this comes from the mother, and if the maximum amount is 5 plus -- obviously, when she is an embryo, the embryo is getting everything from the mother and the mother has a dramatic impact.

At birth, things change, of course, the baby has to breathe on its own, so respiratory gasses go to zero. The mother has nothing to do with that at that point.

For waste elimination, the kidneys and everything sets in, but there is a little bit of control

of the mother. She actually induces a mass reflex to have the babies void and so on. So there is still a little bit of control there.

But those two areas pretty much are on -- the baby is on its own at that period of time once birth has taken place. But physical protection, certainly she has a lot to take care of. She keeps them underneath her. That keeps thermal homeostasis and she still supplies the nutrients obviously up through about day 4.

Then it begins to drop off a little bit. As we get to day 5 and 12, we begin to see that the physical protection begins to drop off and thermal homeostasis, because we begin to develop hair.

And then also the little guys get more ambulatory and start to crawl around and so on. So gradually, these things drop off to zero.

But what we're really interested in and what we are thinking about is what is going to be happening in this area here up to about day 12. It seems to us that one of the important things are the nutrient supply and thermal homeostasis.

Let's see if we can carry that theme forward as we talk about what happens with the data. If we are going to talk about the data, let's talk a little bit about the general experimental design.

We are really interested in what happened with that DNT study. The DNT study sticks out -- it seems to be the study that gives us the most sensitive kind of endpoints. Let's talk a little bit about that.

This is a diagram that tries to depict what the dosing level looks like. And so this time line here -- fertilization would be here. Here is day 6. Here is birth. Here is day 11. Here is day 21 and over here we would have the little juvenile pups.

The thickness of the line here, I don't know why it's red here, this should probably be black, represents the dosing. And so dosing is given to the mother starting on gestational day 6 and that is that thick line.

During that period of time, of course, any dimethoate that's in the mother's bloodstream should cross into the placenta. Because when you talk about placental transfer, basically, everything crosses. It is a question

of how much and how fast and how long it stays there.

So we do have exposure during the intra uterine period. Once birth takes place, we no longer have that connection. And any dimethoate that would want to get into the embryo would have to get there by way of some other mechanism.

The only way I can think of is by way of lactation. Through the milk. We're not sure what happens there. There is no indication that there is much dimethoate in the milk.

And as Dr. Raffaele mentioned a little earlier, the cholinesterase inhibition where -- it seems to indicate there is just not very much. It seems to be a lot less going on here.

And then on day 11, the pups were then directly dosed at the same amount or same dosage that their mother received. And so dosage went back up.

That's the sort of picture we see here. If you look at this, the interesting thing is, of course, that we get pup deaths in the postnatal period in here when we are not sure how much, if any, dimethoate they are receiving.

And when they get direct dosing, we don't have it.

Now, obviously, the pup is developing. Things are changing. But that's just something to keep in mind. Let's look at the next slide.

If we're going to talk about the DNT study, one of the questions we asked was do the pups occur -- normally occur in all groups. And obviously, the answer to that is going to be yes.

And let's take a look at the next slide. And that includes in the controls. Here are three of the studies that we looked at early on. We got the DNT study here with about 25 litters per group.

We had the range finder which had somewhere between with nine to ten litters per group and the comparative cholinesterase study which had 10 litters per group. Now, the dosages are different. You will notice in the DNT we have the control .1, .5, and 3.

The range finder went from control .2, .3, 6. And the cholinesterases went from .1, .5, and 3. The first thing you can see right off the bat is, gee, there doesn't seem to be too much pup death down here in the

cholinesterases study.

As a matter of fact, there is none in the controls and there is only two in each of the treated groups. There is variability here.

If we look at these other studies up here, remember, this dose here is the same as that dose there. It is interesting that we don't get as many pup deaths in here. This is just the number of pup deaths in the groups.

We see that -- if you look at this, you say, gee, well, there is a lot of pup deaths here. This seems to be a concern. And we agree, it is. The question is is that a dose response.

When you look at this at first you say, it is going up. So you want to think about that. And what we want to do when think about that is you begin to say, okay, this is looking at it as if each pup were the independent unit as Dr. Reiss told you.

But in reality, they are in litters. And so maybe another way of looking at it, let's just take a look at the data and see where did the pups die.

The next slide is a display of the DNT study, and I know the writing is small, I think you have copies of this. Each of these panels represents a dose group. Here is the control. Here is the .1 milligram per kilogram dose group. Here is the mid dose, which is .5 and here is the 3.0, the high dose.

Each number underneath here represents one of the dams. And so here is each of -- all the 25 dams for the controls are located here. Here is for the low dose, mid dose, and high dose, and the bars up above it represent the number of pup deaths that occurred in each one.

As you can see, there are litters that had no pups deaths in all the different dose groups. But the black bars are the ones that indicate where pups deaths occurred.

And if we just for the sake of argument say, okay, let's say that the highest level of pup death here, which is three pups, pups dying and the control group is up here, we take that as a background, then we would see that, well, most of the low dose and much of the mid dose

are well within that range.

There is only a few animals that have a lot of pup deaths. But look, there is a lot of pup deaths in mainly three groups, in the three litters in the mid dose group and five litters here in the high dose group.

As a matter of fact, what that tells us is that in terms of the mid dose group 78 percent of the pup deaths, 32 of the 41 deaths that we saw occurred in three litters and in the high dose group 80 percent, 68 of the 85 deaths occurred in those five litters.

That indicates to me at least there seems to be something that is clustered in the litters. We already talked about there might be a biological basis for that. But let's carry this forward a little bit further.

It would be nice if we could take a look and say is there something about those dams that made us say that we could then preidentify them. So we said are there signs of maternal toxicity. What we did was we went back through the data. Recognize this is a post hoc analysis.

We went back to the DNT study, looked at the animal data, started pulling out things. Before we did

that, we said what kind of criteria can we think of that are in there.

We looked to see what kind of observations they made when they did the studies. The studies, the observations they had, they had observations about when the pups were cool to the touch, pups that had poor weight development and there were signs of poor feeding, including if there was no milk in the stomach or the animals look dehydrated.

Now, when we did this, we looked at that, we said let's say any one day when we're looking at this if a litter displayed any one of those signs, we would then count that as an occurrence.

And if a litter had at least two days that had one of those signs, then we said that looked like it might be -- it would be an effect.

Now, understanding this is more or less a hypothesis generating idea, this is post hoc, obviously, there is some bias or possible bias in the way we did this. But we looked at this thing just to see what the pattern would look like. We're interested in patterns.

Look at the next slide. What we did is colored in in green those animals that had these characteristics.

As you can see, it does center around very much the ones that had a lot of problems in the mid and high dose group.

Interestingly, there was an animal that had those characteristics in the control group. And there was also an animal here that had maternal care issues and had no death. I think this bar moved. This should have been over there.

So there was an animal that had what we would consider maternal care issues and her pups didn't die. So that suggested to us that it might be that there are some effects that are being driven by some kind of a maternal care issue.

Also, recognize that those issues we just talked about are ones that are reciprocal. That is to say if a pup is sick, it might not nurse. If a pup is sick, the mother might throw it away. So we understand that. But this is just to look at patterns. We got started with this.

Now let's talk about the cross fostering study. Because the cross fostering study is something that might give us an idea as to whether or not we can look into that.

So the question there is is dam treatment during gestation or nursing -- which period of time might contribute more to that. The way we get at that then is the cross fostering study.

As you recall what happened in the cross fostering study -- the way cross fostering study works, it is kind of a nightmare in a laboratory, you start off like in this case with three dose groups. You have the control, the 3 milligram per kilogram and the 6 milligram per kilogram group.

You get a lot of animals pregnant and you start treating them on gestational day 6. The idea is that when the animals start having their babies, you want to be able to take a control litter and match it up with one of the treated animals that had a baby at the same time.

So if that happens, as long as there is a six hour window, you look for a six hour window only that you

can make those crosses, you can then cross the litters and you can take an animal that was treated by a 6 milligram per kilogram animal and take it over to the control, take the control's litter and put it back on the other side. You have this complicated --

Let's take a look at the next slide. And so we're going to look at the pattern of these deaths. And I'm going to go through a series of slides or series of charts with you. This kind of indicates some things we want to talk about that are different from the ones you saw in the EPA document.

The first one is very minor. There are some corrected numbers from the contract laboratory. It makes absolutely no difference in the statistics. You will see it's only one or two pups per group, but it's the corrected version we used.

We removed in our study the pup deaths that occurred prior to the cross fostering. That's not to say that those aren't important. We're going to come back and we're going to analyze those. But the parameters that you use in a cross fostering study start to be measured after

the cross takes place.

It isn't right to take the animals that died in the control litter before six hours and take that tally and stick it with the cross fostered dam because it wasn't her fault. It is not to say they are not important, and we are going to analyze it, it will be at the end of this, but we're going to look just at cross fostering from the time the crosses takes place.

Then there are two animals we felt were outliers. One of them because she had 23 pups. And 23 pups -- at most, a rat has 12 nipples, usually around 10. And so somebody who is going to starve -- in fact, what happened to this one, she lost seven. And that's what you expect because she ran out of nipples.

But it is not fair to look at that as being a maternal care issue or anything else. So we removed that animal. I'll show you where that is.

There was one animal that was very strange. She was losing weight just prior to birth, which usually you think means she's totally resorbed. But she wasn't. She had 17 pups. The pups were crossed to another dam. And

those pups survived just fine.

But the dam continued to lose weight over the next three or four days and she wasn't feeding her pups. So they had to be sacrificed humanely. We took that dam and that particular litter of the cross out. Those are the two animals that were removed.

Here is the chart that looks pretty much like what you saw in the EPA document. And let me go through this. All the charts are set up the same after this, but let me go through it so you understand.

What we have here -- the characteristics here, this is going to be the dosing region up here. What is in pink is the dosage that was given to the dam.

And recall that the dams are always the animals that were dosed. And so zero means up on the top of these -- this is a control dam, this is a mid dose dam, and those two were high dose animals.

Now, when the cross fostering took place -- those are shown in yellow. The yellow tells you where the litter came from. This litter that is being raised by the control dam came from that dam over here.

So anything that -- this was born of a rat that was treated with dimethoate, but was raised by a control rat and so on.

The two that don't get any different colors are the ones that weren't cross fostered. Dr. Reiss told you about that before. The controls weren't cross fostered. The high doses weren't cross fostered.

That's just the way it was designed. It was a very big study and there were some difficulties.

That gives you an idea what's happening. Now we're looking at the number of litters that are born. And the large numbers of pups and the number of litters is shown in parentheses.

Then we look at the deaths. The deaths are divided up into deaths that occurred between days 1 and 4, days 4 to 11 and then the total from 1 to 11.

And the bottom thing in green here is a percentage, a group percentage of the number of pups. There is number of pups, the percentage, 12 pups out of 375 is 3.2 percent.

So this as it stands here and these numbers down

there again look at the pup as the individual, as the statistical unit.

Now, when we looked at -- this slide shows you where we changed those corrections, and they are shown in green here. This went from 375 to 374.

Each one of these changed no more than one. It changed nothing else. All the other numbers on the table are identical. But our numbers carried this group forward because that's the numbers that we wound up having. It wasn't until we read EPA's report we found out there was a difference.

So now the next slide. This slide removed the pups that were stillborn or nonviable up to the time of crossing. Now, this does change things.

And I don't know why it is out of order, what I did was I blacked or darkened out the mid dose group because I wanted to pay attention to what was going on between the control and the high dose animals.

So what you see when you do this and you remove those, you will see that these numbers down here, the group percentages changed. What we see is, if we go back

to the last slide, go forward, we haven't done that yet, we're doing this. We have taken out -- -- excuse me. I haven't taken out the non viable yet. They are still in here.

What I wanted to do, I want to make these comparisons now between the controls and high dose. Those are the ones I think that are most important as we go through this. But all the numbers appear on your slides.

Let's go to the next slide. Here is where we take out the non viable. You see this went from 374 to 367. This one was like 348. It dropped to 339 and so on across here.

Now we have removed all those non viables and early deaths. We'll talk about them at the end. Here is our starting point. You will see then that the percentage in this particular group here changed.

The others -- this went from 11 to 8.8. This was 7.1, dropped to 3.4 because of the numbers that were in those groups. The next thing we could do is take out those two outliers, removing the outliers.

The animal that had the 23 pups happened to be a

high dose animal. We removed that pup and her litter from the calculations. And the pup that was losing weight before she had her pups was in this group here. It was a control animal. And we removed her as well and also the associated pups that went with that. And those are the changes that are made, makes changes down here.

Now this line up here is the line that talks about the individual pup as a unit. This is the percentage based on -- 5 animals out of 367 is 1.3 percent.

But the more appropriate way of looking at this using litter proportions where you take the percentage for each individual litter and then make that an average, which is the way that the litter proportions are designed, that's what is shown in the lower panel here, that changes it slightly. But it is the more correct way to look at it, we believe. Those are the numbers you get.

If we look at the next slide, we black out the two mid dose groups so we can talk about what is going on between the crosses between the high and low dose group, what we see when look at it is that the control animals,

whether they raised an animal from their own, of course there was no cross fostering there, or an animal that came from the high dose animals, essentially, had the same amount of pup death in terms of percentages. It did not seem to be an effect based upon where the animals came from.

If you look at the high dose animals, the same picture emerges. We have high dose animals that either raised a control or raised their own pups. And you see that the percentage of pups is approximately the same, which suggests to me that the thing that is different between these things isn't really the pups. It is the dosages to the mom.

If mom had no dimethoate, she had a rather small amount of pup death. And if she was treated with dimethoate, there was a large amount of pup death.

It's easier to see this, I think, if you look at it graphically which is the next slide. Here I have taken those numbers and I looked at this in terms of a bar graph. And again, here is the convention we used.

Here the dams were dosed this way. We got the

zero zero. These were the controls. Here is a control that received the high dose litter. Here is a control that used -- the mid doses are in here. And here is the two high dose animals.

Notice that the scale only goes up to 9 percent. But if you look at this, here we go, our two -- the animals when the dam received no treatment, essentially, the same. Actually, for all of these are essentially the -- statistically, these four groups aren't different, but the high dose dimethoate does seem to have a problem.

And we believe that that problem is due to the effect on the mother.

Let's take a look at this another way. Let's ask ourselves a question. If you are so smart, then are these pup deaths occurring in groups the way they did in the DNT?

So we're going to have a scattergram that looks at those again. I only did four groups because it was too small, it got to be too small. We did the high dose and the low dose groups.

We have got -- here is the zero zero. These are

the control litters. Here is the 06. This is the dam. This is the control. And her litter was from this group down here. So she is raising a dimethoate group.

Here is a dimethoate animal raising a control litter and a dimethoate animal raising a dimethoate litter.

If you look at this, once again, each number underneath here represents an individual dam and the bars represent the number of deaths in each litter. You can see that there is a scattering of these among the lower groups.

But as you get to the animals treated with dimethoate, there are more of them and in some cases higher amounts.

You can say to yourself, well, they aren't as big as they were in the last one in the DNT. But part of the reason is, remember, you had the six hours of time, we lost some pups in there and those aren't on this thing.

The next question, then, was are there any maternal care issues. Having designed this thing a little more prospectively, the contract laboratory was able to

say there are some things they are going to look for.

The things they are going to look for are listed on the next slide. They are looking for scattering of pups, restlessness of the dams, physical abuse of the pups, where the pups were cool to the touch and whether or not there was no milk in the stomach.

We used the idea there was at least two pups. Admittedly, we're looking for patterns again. I'm going to point that out. We're not looking for specifics. In retrospect, there are probably a lot of better ways that this could have been designed to make it more quantitative in some respects, but the patterns are really striking when you look at all of the raw data.

What we did with this was we said, okay, if you have one of these characteristics on a given day, that's an occurrence. If you have 20 of those on a given day, it's one occurrence. That is the first thing.

Second thing is you had to have at least four of these occurrences in order for us to think that there was a maternal care issue. The reason that came about was, my fault, we knew that they looked at animals four times a

day in the cross fostering study and basically once in the DNT.

So in order to try and make it more or less even, I figured four times, four times the amount, because you have more opportunities of catching it. So a random time when you look at these. That's the number we used.

Now, it turns out that we did a post hoc analysis of this that if you looked at three occurrences per day, there is a lot of these things in the controls too at about three times, three occurrences. Because -- although, rats are really good mothers, they are not perfect. Sometimes they move their pups around in the nest or in the cage and if the technician comes by and sees they are scattered, well, they are scattered.

He doesn't know if they are scattered because she is rebuilding her nest or if they are scattered because she's just a terrible mom.

We looked at that. We used four as the number. Again, we looked at that and colored things in in green. What we saw was that we found a lot of the maternal care issues occurred in animals that were in the dimethoate,

high dose dimethoate treated groups.

There is one here in the control group that nearly all her pups died. Again, the large numbers in red represent those animals that had maternal care issues, but had no pup deaths. There are quite a few of them.

It is not perfect. But the pattern does emerge. And there seems to be something going on with the pups -- litters, rather, the dams, when they are treated with dimethoate.

Even the ones here where they had pups that survived, there is more of those animals that had these maternal care issues in the dimethoate treated moms than in the control moms.

By the way, this does carry through for the mid dose. But it is not shown here.

Now, so that's our picture on that. But I told you I wanted to come back to this early pup death. What happened in the first six hours.

So we go back to the first six hours. You have to recognize to do one of these studies, admittedly, if you look at those things, you say, wait a minute, some of

those numbers, the 374 dropped to, what is it, 369 or something. There were some pretty big drops in there. What is going on.

You have to recognize that when you do these studies, in order to have these banks -- you can't guarantee they are going to have their pups all at the same time, there are a lot of animals in this study.

Records were kept on all these animals up until the time when the crosses were made. This includes the animals that were not included in the crossing.

The next slide shows how many litters there were to start out with. In the controls, there were 71 litters. In the mid dose group, there were 23 litters. That was the one that only had one group. There were 45 animals that were 6 milligrams per kilogram dimethoate treated animals.

For that 6 hour period of time, if you compute the litter proportion of pup death for each of those groups and notice that the scale goes from 0 to 10 percent, you wind up with the controls had 1.8 percent and the high dose had 2.4 percent deaths. These are not

statistically different.

Even looking at this thing where it is an expanded scale to only 10 percent, there is not much of a hint, much going on there.

What must have happened in this case, it is just one of the -- it is the luck of the draw, I think, that the animals that were selected to be put into the crosses weren't selected by any other reason that they had to be born at the same time.

And remember we saw the clustering of the death in these things? The clustering occurs in this 6 hour periods too. It just happens that the ones that got put into the experimental design are the ones that had some of the pup deaths, and the number of the ones that didn't have pup deaths in the first six hours weren't put in there.

I think that's what the next slide says. That's concluding remarks.

We agree and we certainly concur that the pup deaths are a very important endpoint of concern for dimethoate. But we point out and really hold strongly

that the maternal animals do exert an influence on their litters, and, therefore, they have a possibility of having a strong influence on pup death.

I believe it is a biologically credible statement and certainly it is evident by what we have seen in the distribution of the pup deaths as we saw in the slides previously.

We believe that the pup death is associated predominantly with the repeated exposure of the dams to dimethoate during lactation, that the gestational only exposure has minimal to no effects on the offspring health.

You have to go back and think about this. We talked about animals that had the 0, 0s and 0, 6s. Remember? We looked at those animals that were controls. They had basically no effect.

They were the same whether they came from a dimethoate treated mom or from their own mom. The amount of pup death was the same in those.

When we went to the high dose animals, again, it didn't matter if the litter came from a control animal or

from a dimethoate animal. The amount of pup death was the same. It looks to me like the gestational thing does not seem to wash in this particular design.

It looks like it is lactational effect. We also believe that most robust analyses are the ones that Dr. Gaylor and EPA has used which is the benchmark dose approach.

And that because of the way it is computed, it does take into account both the maternal influence and pup variability. I think it would be -- it is another step in the direction of strong science to include multiple studies where possible in order to get more confidence in the data.

That's it.

DR. ROBERTS: Thank you, Dr. DeSesso, for your presentation. Let me ask the panel if they have any questions for you. Dr. Collins, then Dr. Cory-Slechta.

DR. COLLINS: In looking at your --

DR. LI: Just to let you know, we're going to have both Dr. Carl Keen and John DeSesso here so they can field your questions together.

DR. ROBERTS: Thank you, Dr. Li.

DR. COLLINS: In your pup deaths during gestation you say there is more influence during the period of gestation. Upon looking at your teratology studies, which we didn't see, I gather that they have been looked at, were there effects in the implantation loss, when one did the teratology studies?

DR. DESESSO: No. The teratology studies were pretty clean. I think --

DR. COLLINS: Would replicate your gestational -- assuming they were done at the same dose levels.

DR. DESESSO: Yeah. Well, the teratology studies were clean. Actually, I think the high dose was higher. There were no malformations. There was no increased incidences of stillbirths.

And Dr. Li will be going over that in her next paper as well.

DR. COLLINS: Sorry.

DR. DESESSO: That's okay. The point is that we did look for those things as well.

DR. ROBERTS: Dr. Cory-Slechta.

DR. CORY-SLECHTA: I'm still a little troubled by some of the terminology. I guess could you define for us what dehydrated appearance is in terms of how somebody would know that and measure that and what about physical abuse?

DR. DESESSO: Physical abuse is easy. They were chewing on the pups. So you have bite marks and that's --

DR. CORY-SLECHTA: Right. But what about dehydrated appearance?

DR. DESESSO: Well, the animals begin to look shriveled. Basically, the animals are starting to die, I think, on most of those.

DR. CORY-SLECHTA: How shriveled? What constitutes dehydration? And my other question would be if this was done four times a day, how long was each dam and litter looked at given the number of pups that you are talking about, and was it done systematically across all treatment groups and all litters?

Because I find it a little unusual that the only group that didn't have a maternal care issue at least as

you have defined it was the controls.

As you have even pointed out, it does happen in controls.

DR. DESESSO: There were some. They were the big numbers at the bottom. They just didn't have pup deaths associated with them.

DR. CORY-SLECHTA: Could you tell me about what the time frame for evaluating each dam was, whether it was done across each treatment group and consistently and systematically or were these random or how was it done?

DR. DESESSO: All I can tell you is what is in the report. We can ask Dr. Hazeldon about that. But they were done four times a day. Their cage side things were -- it sounds like the -- the technicians went through the room and checked each cage, looked for the things they were told to look for and recorded it. That's as systematic as you can get, I think.

One thing you have to recognize, the logistics of these things, should be perfect and they aren't always perfect, but this is an enormous study. Think about how many pups there are in all of this.

They are doing the best they can with trying to make these observations. The whole business about the maternal care issues as we're defining it now -- these were criteria that were separate.

We weren't looking for maternal care -- they weren't looking for maternal care issues when they did this study. As I told you, we got involved in this in July. The studies were already completed by the time we saw the data as well.

You can say, well, you can design it differently. It is true. Some of this stuff is post hoc. But I think what strikes me is the patterns. If you look at the raw data, what you see is it isn't like we're cherry picking anything.

Some of these animals that have these -- they have 8, 9, 10 days worth of these observations. And ones that only have two or three, including the controls, if you are looking for less than four observations, you have plenty of them in controls.

So there seems to be a cutoff. Obviously, like I said, animals are -- the rat happens to be a very good

mother, but they are not perfect. So of course you are going to get these other things.

I understand you are skeptical, and I understand. I'm skeptical too. That's what we do. But we're making the best interpretation of this I think we can given the data as we see it.

DR. ROBERTS: Dr. Foster.

DR. FOSTER: So John, we didn't a chance to see the teratology studies. Were these conventional teratologies dosed up to GD 20?

DR. DESESSO: Yes. Up to GD 20? Wait a minute. Were they GD 20 or 15? They were 15.

DR. FOSTER: These are not directly comparable. Because as you know, you can have effects that are occurring later on in gestation that you wouldn't pick up in a teratology study.

DR. DESESSO: Yes.

DR. FOSTER: So I think we have to be a little bit careful about jumping in and saying that there is no gestational effect here.

I think the other thing that occurred to me is

when you start to see pups dying in the first one or two, three, four days after birth, that raises all kinds of other issues, based on the Chernoff Kavelock assay (ph) and so on and so forth.

So I'm not sure how you can say these are maternally driven rather than some kind of in utero.

DR. DESESSO: Well I --

DR. FOSTER: You could just as easily build the other argument, that it is actually some event that has occurred in utero that then manifest itself and the mothers don't want to nurse sick animals.

DR. DESESSO: Okay. If we go back. Let's take a look at this. Here is something we can talk about.

When you look at the cross fostering and you start seeing -- when you look at this, you say, okay, here we have animals that came from mom over here that were treated in utero and they are raised by a controlled dam and they don't seem to have tremendous amount or -- anymore deaths in them than do the ones that are controlled that raise their own.

It is the only piece of information we have. But

the thing is nothing jumps out at you right away saying, wow, look at that. That seems to be an issue.

DR. FOSTER: That's a control.

DR. DESESSO: This is a control animal that had a dimethoate treated dam -- litter come over here. So this litter was gestated here. And whatever was in there that should impact it, oh, I'm a sick pup, came over here and now it is being raised by a control dam and things look pretty normal.

This is only one data point. But I don't see another interpretation to say that -- if what you are saying is true, the pups that come out of here, if they are weak or sick, ought to have some problems if they were raised by a control animal. And I don't see it.

I take this animal here that was gestated as a control and put her with an animal that's treated during lactation, and I do have problems.

Now, the one thing you could catch me on, but I'll give it to you, you say, wait a minute, John, what is going on here between day 1 and 4 and 4 and 11.

Although, the total number of deaths are

approximately the same, they seem to be reversed. What could be going on there. By speculation, granted. But although these guys are being raised by a dimethoate animal, they spent the first six hours with mom.

And mom fed them the first time. Whatever colostrum the rats get and so on were given to them before they came over here.

It may give these pups a little bit more of juice, not juice, but a little more vim and vigor that they managed to last beyond day 4 before started to die.

What I don't have and maybe it would be interesting to look at is see exactly when they died. Like if these guys all started dying like on day 5 or 6, then you would say, well, then, that would make some sense.

When I look at this, and I was thinking, well, the bottom line here seems to be the same, the differences in there, I think, could at least be explained by that little piece of biology. But it is speculation.

DR. ROBERTS: Dr. Cory-Slechta.

DR. CORY-SLECHTA: I just want to raise one point. Your 1.6 figure for that dam 0 foster litter 6 of

course is corrected. You have to essentially assume that the removal of those were legitimate.

That's a different figure than what we saw before. We have to buy into your presumption.

DR. DESESSO: Go back one slide.

This is what we have in here. The animal that -- this is the animal, there was a control animal. She is the one that was losing weight repeatedly.

She delivered seventeen pups. The 17 pups went over here and lived. She received 19 pups from here and continued losing weight. So she was sacrificed humanely because that's what you do.

You can grin. But the thing is -- she was sick and was dying. How does the dimethoate -- how does the treatment of the pups over here affect that? This was a decision that was made early on.

It wasn't because the pups were dying yet. The pups were going to die because she couldn't nurse them. She couldn't carry them. The alternative, I guess, would be to take the pups out over here too maybe, the ones that survived. You see what I mean?

But even if you leave that one in, there are numbers here, 3.4, it is about -- it's just a little bit larger than the mid dose group, it really still stays within the same range. It is nowhere near what it is over here where you have the high dose animals, high dose dams.

DR. ROBERTS: Dr. Pessah and then Dr. Harry.

DR. PESSAH: Was there any attempt to see if there were any patterns for the pups that survived in terms of behavioral abnormalities?

DR. DESESSO: In the cross fostering?

DR. PESSAH: Yes.

DR. DESESSO: Not that I know of. No.

In the DNT there was, in the developmental neuro tox there was.

DR. ROBERTS: Dr. Harry.

DR. HARRY: A couple points for clarification. The controls were maintained with their -- the 0 0 was maintained with their original litter.

DR. DESESSO: That is correct.

DR. HARRY: And the 6 6 was maintained with their original litter?

DR. DESESSO: Yes.

DR. HARRY: So you minimized the stress factor. You didn't even cross -- you didn't foster within their --

DR. DESESSO: That's true.

DR. HARRY: And, of course, you could do a split cross foster, fully randomized model and address a lot of these questions that Dr. Foster has brought up. But talk about another nightmare.

The other question that I wanted to have, which is really bothering me in this whole study, was the number of pups per litter. These are very large litters. Seventeen.

DR. DESESSO: Yes, they are.

DR. HARRY: I guess I will address you as well as your group over there.

What effect does it have on -- if you are stressing the mom and you are going to have maternal effects, what type of confounder does it present when you start then adding the stress of having these huge litter sizes in there?

So you are having more than 10 or 12, which is the whole justification for culling for these studies in the first place. So can we get any feedback from you guys and the experts of what impact that might have to feedback in on interpreting some of this data?

DR. ROBERTS: Please identify yourself.

DR. KEEN: This is Carl Keen.

In terms of the maternal toxicity effects, you can show that the effects on the prenatal side will be more severe. And that's expected because a lot of the effects associated with maternal toxicity is interruptions in nutritional delivery to the conceptus or else to the neonate.

You will see an amplification often times. You will see more even subtle defects in malformations that you contract to the -- as opposed to what the initial stressor was.

Postnatally there has been less work in this area, but the limited amount would suggest it is there. It is not quite as strong.

For example in some of the classic work looking

at postnatal maternal toxicity, which was just simply food restriction, that was done in the early 70s by Ziemans Group (ph) and later by Kavelock's Group at EPA.

Even when you then brought litters down to four or six pups you would still see distinct differences. So you don't lose it. You can magnify it if you have very large litters.

In my opinion, that is only a significant issue if the litter size is markedly different across the groups, which was not the situation here.

DR. DESESSO: The other thing too is in terms of the culling is that that's always been a raging thing for -- as you know.

If you cull, people think you lose information. If you don't cull, they say, well, everybody is weaker. It is sort of -- you have to pick one way and do it.

I guess they picked the way. They didn't cull.

DR. ROBERTS: Dr. Harry, do you have a follow up?.

DR. HARRY: Yes, I have a follow up. Is there data available on the body weights? You would expect that

if you have got problems with maternal behavior, that you would also start to see some body weight changes in these pups.

Did you have any to analyze within this?

DR. DESESSO: Keith, did they measure body weights on a daily basis?

MR. HAZELDON: The question was was there any kind of selective effect on, so parallel effect on the body weight on these dams. And in this study, there wasn't.

DR. KEEN: I think it is worth noting that in some cases of what we call maternal toxicity postnatally, again, typically secondary to low food intake because that's what has been studied, and that's not the situation here necessarily, you don't necessarily see a dramatic effect on pup weights.

The first 24 hours can really set the stage. What John referred to is kind of a hypothetical situation where he said is this first six or eight hours of milk actually that critical.

To my knowledge, it's very thin data when it

comes to rat models. On the other hand, if these were mouse models, there is very good information going back to the mid 1950s.

Isha Murra (ph) was able to show that the very first half day of suckling provides sufficient colostrum. He wasn't sure what it was. We think that was probably zinc based on work done with a lethal milk mouse. Was sufficient to completely protect that mouse against acquiring zinc deficiency later.

So there is something. It is not just -- it is colostrum. But within that first to six to eight hours, the milk composition is fundamentally different.

You can have an interruption in that or an alteration later that would not necessarily translate into a change in body weight.

MR. HAZELDON: I would like to clarify what I said just a minute ago about the weight gain. I'm just looking at figures for the weight gain for these mothers on this study.

And there is overall during the latter part of gestation we looked specifically at the very end of

gestation, day 17 to 20, there is no formal effect across any of these groups in terms of gestation weight gain over that specific period.

But then when you look at lactation weight gain in the first four days, lactation 1 to 4, there is a trend for reduced lactation weight gain in these animals which received 6 mgs per kg.

It is reduced compared with the other groups. There is not much in it, but it does exist. Particularly, in the group where the animals have their own litters, the 6 and 6.

So that trend does exist. When you look then subsequently to that, days 4 to 11, again, there is no real difference. It is just those first few days.

DR. ROBERTS: Thank you for that clarification. I think Dr. Reed has a question.

DR. REED: This is sort of a follow up question for Dr. Keen.

If I heard you correctly, you said that the possible influence of having a larger litter is that it would up or emphasize the pregestational effects. Right?

Could it be that it is also possible that it is the other way around, that it raises the incidence of pup death in the controls and thereby sort of mask the effect of prebirth defects with the pups.

DR. KEEN: It is possible. Certainly if you take a look at just litter size as a predictor of postnatal mortality in control animals, once you get litter sizes above 13, 14, 15, routinely, you will see a higher frequency in postnatal mortality.

Again, that's a simple supply and demand argument, much as Dr. DeSesso indicated.

On the prenatal side, in case my comment wasn't clear, particularly if it's maternal toxicity that is causing an interruption or an alteration in nutrient transport across placenta, because we think that's one of the primary mechanisms, it is simply the larger the embryonic fetal mass, the more you are going to see an impact of what is already a reduced delivery.

So that part is fairly straightforward.

Postnatally, if what you saw in the first two or three days was maternal toxicity affecting her ability,

either her desire or ability to provide milk, and on the human literature we have a lot of indications that there is maternal stress, that often times this is a social with lactation failure in the first few days, well, then, again, the larger the litter, in theory, you would argue, well, that's going to increase the probability of seeing early postnatal loss if it is simply an energy or nutrient delivery issue.

DR. REED: I guess a clarification on this again is that regardless or actually exclude the possibility of some effects of dimethoate on the maternal, I mean, when you look at the 0 dose group, again, just because you have larger litter size itself, wouldn't it also affect the possible incidence of pup death because of the large size of the litter and so that it raises the background level, is what I'm thinking of, of the pup death because of the size of the litter?

DR. KEEN: In the control group, you would expect that you would see a large -- the larger the litter size, the higher postnatal mortality.

To your point, yes, the background would be

higher.

DR. REED: So it's possible.

DR. KEEN: Given the fact that litter size was not dissimilar across the groups, I would argue that the same background level that's being driven strictly by litter size as opposed to any additional putative effect by the agent being studied is kind of irrelevant unless the change in background is so large that you would run the risk of swamping out a small effect.

But that's not what I see in these data sets.

DR. REED: Thank you.

DR. ROBERTS: Dr. Pessah, then Dr. Harry.

DR. PESSAH: I have a more general question. The choice of the strain for the study, was that based on any particular set of criteria? Are they more sensitive or more resistant to OP toxicity than other strains?

MR. HAZELDON: The choice of strain is just based on our experience with the particular rat in that laboratory. It is a conventional strain which is used for all of the reproductive studies in our laboratory and in many other labs in the UK.

DR. PESSAH: Is there any indication that it is more sensitive or a susceptible strain or a resistant strain to OP?

MR. HAZELDON: I have no data on that.

DR. ROBERTS: Dr. Harry had a question as well.

DR. HARRY: It is a rather quick one, but it's trying to compare the different studies.

If you did the developmental neuro tox study, you do cull the animals down. Again, granted you are looking between the one to four for the major component of death. So that should have covered both of them.

If all of those litters between both studies had the same number of pups within them, then you would expect it to be relatively equal across studies to determine mortality on the stress of the animal and the litter size effect that might be there within the one to four day window.

But if you looked from the 4 to the 11 day window, then you would find a difference between these two studies because of the number of litter pup size potentially.

MR. HAZELDON: Yes, you would. Although, one thing that I would like to observe is that these rats have been producing these large litters for a long time. They are tooled up to -- they are quite capable of raising these fairly large litters.

And what you find is there's quite a wide range of litter size over which there is very little variation in terms of background litter death.

It is only at the extreme range of -- extreme end of these ranges that really this kind of kicks in.

The curve, if you like, is quite a flat central portion of the curve. And when you get to litter sizes of, say, perhaps 16, 17 or higher, that you would expect to get more death, and below that in which almost all of these litters sit within that range, there is actually very little variation across that wide range of litter sizes as regards the incidence of litter death.

You can't actually draw a very nice straight line through it. There is a great cloud of points. It's only really at the extreme end of that range where this excess death related to litter size will actually occur.

DR. HARRY: I have a follow up.

I guess my point was more if you were dosing the dam with a compound that could influence in a generalized aggravation to the dam, I'm not saying toxicity, because quite often we look for gross toxicity, but we influence her maternal behaviors -- and we know that that can happen.

So if we influence her maternal behaviors, we may shift that curve such that now it isn't 17 she can take care of. It may be 14 she can take care of.

So I guess my point was when we're trying to look at the differences between the developmental neuro tox study, which again was one of the reasons for culling those animals down to a lower level, and you look at this study where you maintain larger litter sizes, could you view this study in such a way that you think you may actually be stressing the system more, that you may be unmasking more effects that might have been there in the neuro tox study but you didn't see because you had smaller litter sizes?

MR. HAZELDON: That seems credible.

DR. KEEN: I would argue that the cross fostering study, the way it was designed, is far more likely to really reveal if there was effects occurring on either side, either prenatally or postnatally.

What hasn't been commented on too much is, again, the observation that even direct dosing of the pups later when you are outside of this first 3 or 4 day window basically had no impact on their survival.

The day 3, I have heard a few allusions that it is not that it is magical. If you were to look at milk composition, it fundamentally changes around day 3, day 4.

Effectively, colostrum is one thing on day 1. But between approximately day 2 and day 4 is where you have marked reductions in the concentrations of a number of nutrients.

And we also know that the entire mammary process during that time period changes. But if you have a very small litter as a consequence of that, day 4, day 5, and if you have a limitation of nutrients, you may not see it.

Or you would see it if you had a much higher litter.

DR. ROBERTS: Any other questions?

DR. LI: You put a slide up. Did you want to --

DR. DESESSO: I want to make a comment. The slides we gave you, there is a misprint on it. It's slide 20, the last line. I think yours says aggressive dam. It was supposed to say abnormal dam, weight loss prior to birth and postpartum.

This is that dam we were talking about that was in the infamous group that Dr. Cory-Slechta and I liked.

But she wasn't aggressive. She was losing weight. I wanted to make sure -- if you look at this tomorrow and you go, when did we talk about that? It's because we didn't.

We talked about the one that lost weight.

DR. ROBERTS: Last call for questions before we break for lunch. Seeing none, thank you very much, Dr. DeSesso, Dr. Keen, Dr. Hazeldon, for your presentation and responses to our questions.

I would like to break for lunch for an hour. Let's reconvene at 1:30 sharp to continue with the public comment section of our agenda. Thank you.

(Thereupon, a luncheon recess was taken.)

DR. ROBERTS: Let's continue with the public comment period and continue with the comments from the Cheminova group.

We're commencing now with a presentation by Dr. Li.

DR. LI: Thank you. As promised, I'm the last presentation. I'm going to try to cover two subjects.

I'm going to give you a brief overview of the weight of evidence from some of the other reproduction studies. And then I will wrap up with highlights of the key conclusions from the previous presentations that we think are especially important as you consider the risk assessment for dimethoate.

And then finally, we are going to review the EPA questions to SAP and give you our brief response to those questions based on our analysis.

So the weight of evidence of the other reproduction studies. So earlier today, Dr. Raffaele noted that the weight of evidence from some of the dietary reproduction studies indicated that there were effects on

pup mortality in these other studies.

And that that supported the conclusions about the pup mortality in the DNT study. And we agree that it was really important as we were evaluating this pup mortality that we should look carefully at the reproduction studies.

And even though there is a difference in terms of what one might know about the dose, there is a similarity in that from postnatal day 1 through 4 where most of the deaths occurred in either of the experiments the pups never received a direct dose.

So it is really worthwhile to look at the different reproduction studies.

Before proceeding, I think what is important in evaluating the pup mortality is to first review what was seen in the DNT study. Because there was a certain specific pattern of effect.

And when we look at the reproduction studies, we should look at them in terms of whether there was a similar type of effect as that seen in the DNT study.

So in the DNT study, what was seen was that both

pup mortality and cholinesterase inhibition were critical effects. And what that means is that both of them were the most sensitive endpoint in the DNT gavage studies.

The other thing that was different is that, although we believe that there was a strong maternal influence related to pup mortality, there was no overt maternal toxicity.

So let's now look at the different studies. In the first study, this is a 1990 one generation dietary dimethoate reproduction study. This is one of those range finding studies in which you purposely tried to dose at higher doses to try to figure out that highest dose below which you will get severe maternal toxicity.

And in that study, there was in indeed increased pup mortality. But what is different is that that was associated with overt maternal toxicity. For example, there were tremors throughout lactation.

There was also significant cholinesterase inhibition at all of the dose levels. And I got the numbers out here. So at the lowest dose level, we're talking about 53 percent cholinesterase inhibition. At

the mid dose, 60 percent. And at the high dose where there was increased pup mortality, there was 68 percent decrease in cholinesterase inhibition.

So unlike in the DNT study, the pup mortality in this one generation study was not the critical effect. And it was seen along with overt toxicity.

In the second study, which is the more complete two generation dietary dimethoate reproduction study, there was no increased pup mortality. We agree with EPA on their assessment on that.

Yet there was significant cholinesterase inhibition. As much as 68 to 69 percent inhibition in the high dose. And again, so pup mortality is not an effect. It is not the critical effect.

The next study is another two generation dimethoate study conducted in 1992. Again, we agree with the EPA that there was no increased pup mortality. But there was a possible decrease in number of live births per litter in the high dose.

And just to be complete about the weight of evidence, that decrease in number of live births was not

seen in the more recent 2003 two generation study.

Never the less, if we consider that this highest dose is an effect that's related to the pup mortality, there was substantial cholinesterase inhibition at the high dose and at the mid dose.

And so different from the DNT study, pup mortality is not the critical effect.

There are two studies with omethoate. And the first one is a 1992 two generation omethoate drinking water study.

There was increased pup mortality at the high dose in F 2 A which was associated with overt maternal toxicity during lactation in which there was decrease in food and water consumption and body weight gain.

This is different from the picture that we see with the DNT study. And in fact, there was significant brain cholinesterase inhibition which was 55 percent at the highest dose, 30 percent at the mid dose, and 16 percent at the lowest dose.

So unlike the DNT study, pup mortality was not the critical effect. And what you see in fact that in

these dietary studies, you don't have to do much BMD analysis or quantitative analysis.

You see that there is a larger differential between the dose at which you see pup mortality and the dose at which you are seeing cholinesterase inhibition, the doses at which you don't see cholinesterase inhibition.

So in the final study that was available for omethoate, this was a three generation dietary study, there was an increase incidence in pup mortality compared to controls at the two highest dose levels in the second generation. Not in the first and the third.

And there were no clinical observations made. This was a really old 1981 study. And when we looked at the methods section, it was really not adequately described up to the standards that we expect today. We couldn't tell what they did and when they did it.

And cholinesterase inhibition was not measured in the study. So basically, we really can't make any kind of conclusion from this particular study.

And just as a piece of information, the studies

with omethoate have to be considered a little more cautiously than the dimethoate ones. Omethoate is about a one to five percent metabolite. One to five percent of the total dose administered to the pup -- I mean to the dams of dimethoate.

Only one to five percent is omethoate, and that's found in the urine.

And we don't really understand what is the internal dose of omethoate following dimethoate. And so I just say that in terms of the overall weight of evidence, we looked to the two generation dimethoate dietary reproduction studies, and there is no evidence of increased pup mortality in either of these two generation studies.

There is no consistent evidence of a decrease in the number of live births per litter. And in those cases when there is increased pup mortality or decrease in live births per litter, they were not the critical effects. There was substantial cholinesterase inhibition happening at lower doses.

So that was basically the summary of the weight

of evidence. There was a question earlier about the teratology studies. And basically, the way we felt about it is that the reason to look at that carefully was just to make sure there wasn't anything really happening seriously during gestation.

But we cannot -- we didn't feel comfortable to use that study as proof for anything that was happening postnatally. Because sometimes functional effects are far more evident than just looking at a teratology study.

So our main reason to look at the teratology study was just to make sure that we could say that it was clean. And it was clean in the dimethoate study.

So now I'm going to the last part of my presentation. And I would like to summarize for you the key conclusions from all the presentations that you have heard.

From the biological, more qualitative analysis of the data, we see that there is a strong maternal influence on pup mortality.

There were signs of maternal neglect which were associated with litters in which excess pup losses

occurred in the DNT study.

And we recognize that the way observations were done is not like what we do in an FOB on a neuro tox study in which you have a whole list of criteria that you are able to rank everything.

What I would like to point out is that they actually looked at the dams and the litters together five times a day. And they want to make sure that they are not disturbing the dam and the litter too much.

So they open and look at them to the extent possible that they can mark the five different types of observations. They do it to the best of their ability.

But sometimes you can't see all the pups. It sort of depends on how they are laying. It is not perfect. But it shouldn't be thrown out. The data shouldn't be thrown out.

In order to try to compensate for some of those limitations, one of the things I was concerned about is that many of those observations are really closely related.

So, for example, if you have a lot of scattering

of the pups, well, the pups may be cold to touch. So what we did to try to compensate for that is if there were several observations that were made on one day, we only counted that once.

So in other words, what we were seeing is that there usually was a pattern of different effects happening, but we only would say that that animal had one incident of that occurring by just counting it one day.

When they had four or more days, that was sort of our arbitrary cutoff. And that is how we evaluated the data. And as we discussed earlier, there were control litters that had up to one to three incidences, that is days, of maternal toxicity.

So although it wasn't perfect, we tried to do some things more conservatively to try to compensate for some of the limitations of the data.

We cannot prove that there is a mechanism for maternal toxicity causing pup mortality. But when you step back and look at the data, there is a really good association.

The cross fostering study revealed that the

increased pup mortality was associated with maternal exposure that occurred during lactation and that gestational only exposure had minimal to no effects on risk for pup mortality.

I think John DeSesso went through that very carefully this morning. And I hope that you will look carefully at the data in terms of who was -- what dose the dam received and how that affected the pup mortality.

So the implications of this more qualitative weight of evidence analysis is that because adult brain cholinesterase inhibition is the most sensitive endpoint, that protection against that would protect against pup mortality in our opinion.

The key conclusions from the BMD analysis was that when we compared the BMDL 10s for brain cholinesterase, we found that the BMD 10s for adults were lower than for the fetus and for pups for comparable periods of exposure.

When we then compared the brain cholinesterase inhibition with pup mortality, and now since we know that adult cholinesterase inhibition is the most sensitive,

when we compare the adult cholinesterase inhibition, the BMDL 10 with the BMDL 5 for pup mortality, we see that we get a 2 to 3 fold difference using the meta analysis, all of the available data following gavage dose and that this difference becomes even larger when one looks at the dietary route of exposure.

I would like to point out that the BMD is really a quantitative way to take a weight of evidence approach of all the DNT and related gavage studies.

This approach is, in fact, consistent with EPA evaluations of other chemicals. Just earlier this year in August, EPA issued an IRIS monograph in which they derived an RFD based on the BMD from developmental toxicology studies.

There were three of them that they combined all the data together to derive a BMDL O5. So what Reiss and Gaylor did is very consistent with the practices by EPA.

The implications of this BMD analysis for the risk assessment is that adult brain cholinesterase inhibition can be used as a point of departure for risk assessment for repeated exposures.

So our conclusion from both the quantitative and qualitative approaches to looking at the data are that brain cholinesterase inhibition in adult rats is the most appropriate critical endpoint for risk assessment and is protective for pup mortality.

And as I said, this conclusion is supported by both the BMD analysis and our more biologically based qualitative weight of evidence analysis. So now for the questions to the SAP.

The first question asked, please comment on the information available for dimethoate which characterizes the underlying causes of the pup mortality in the dimethoate DNT study and the degree to which this information can be used to determine the impact of maternal neglect, maternal toxicity on pup mortality.

We believe that the data from the DNT study and the cross fostering study together show that there is a strong maternal influence on pup mortality.

The second question asked whether the results of the cross fostering study suggests that the pup mortality observed at lower doses in the main DNT study may be

attributable to a single dimethoate exposure.

And we believe that the weight of evidence is that pup mortality is strongly related to postnatal maternal behaviors and not a direct effect of dimethoate during gestation.

I want to point out that even without considering the maternal behavior, if you just look at the numbers from the cross fostering study, there is clearly a postnatal effect and there doesn't appear to be a gestational effect.

After consideration the results of the BMD analysis for brain cholinesterase inhibition and for pup mortality, it is proposed that brain cholinesterase inhibition be used as the endpoint for the dimethoate risk assessment for all durations.

And that this would be protective for the pup mortality. And we agree.

We believe that the risk assessments based on cholinesterase inhibition will indeed be protective of pup mortality. We believe that the pup mortality is associated with maternal behavior following repeated oral

gavage exposures to dimethoate.

The quantitative BMD analysis indicate that adult cholinesterase is protective of pup mortality based on gavage studies and that the dietary reproduction studies provide even stronger evidence that adult cholinesterase inhibition will be protective of pup mortality. And dietary is a major route of exposure.

Direct exposure to -- and I also wanted to note so that we don't forget this point, that when we dosed -- when the pups were dosed directly from postnatal day 11 through 21, this did not cause an increase in pup mortality.

And that this pup mortality occurred during a period when there was questionable exposure through the milk based on the cholinesterase levels that were found in the postnatal day 4 pups.

So that concludes our remarks. I would like to invite the rest of our panel up to field additional questions that the panel might have. Thank you very much for your attention.

DR. ROBERTS: Thank you, Dr. Li. Let's see then

if the panel has any questions on either Dr. Li's presentation just now or if any other questions have come to mind based on some of the earlier presentations before lunch.

Dr. Harry. Dr. Harry, you can direct your question actually to whomever you think --

DR. HARRY: Whoever is sitting in that corner.

DR. ROBERTS: Somebody who used to be sitting in that corner will need to answer this question. Dr. Keen perhaps or Dr. Hazeldon, maybe?

DR. HARRY: I had two questions for whoever knows the information.

One of them was a presentation that Dr. DeSesso had given where we were doing the differences that were happening in the dose ranging study for the DNT, the DNT and the cross fostering study.

And you had very different things that were happening with the six milligrams per kilogram dose as far as pup mortality, making the DNT study look relatively aberrant.

I'm making the assumption that all of the

chemistry of all the dosing solutions and everything else confirmed that they were equivalent doses that were being delivered to. So the chemistry supports that that dosing solution was exactly the same between those studies.

DR. LI: The studies were conducted by GOP. That's one of the requirements, yes.

DR. ROBERTS: The response is by Dr. Li.

Since there is going to be perhaps different respondents to Dr. Harry's question, if you could identify yourself for the record, it will make it easier for the recorder to figure out who is responding. Thank you.

DR. HARRY: One more.

DR. ROBERTS: Please continue.

DR. HARRY: The other one was based upon a slide that you put up about -- you made a statement, the cross fostering study revealed that increased pup mortality was associated with maternal exposure that occurred during lactation.

I don't think your cross fostering experimental design allows you to make that statement because you did not have a dam that was only exposed during the lactation.

So it's a technical point, but it is something that it really -- it reveals that you had a continued dosing and that something was coming across with the maternal dosing, but not necessarily that it was the dosing during the lactation.

DR. LI: Yes. We were trying to figure out better ways to communicate that without making the sentence twice as long. But I understand your point. You are absolutely correct.

DR. ROBERTS: Dr. Cory-Slechta.

DR. CORY-SLECHTA: In trying to make the case to separate or essentially to link, if you will, maternal toxicity to pup mortality, you showed us data from a 1990 one generation study in which you said that increased pup mortality was associated with overt maternal toxicity, for example, tremor.

Tremor is a very difficult thing to measure in rodents. Was it actually measured or are we talking about clinical systematic observation?

DR. LI: No, they are not like the FOB. They are standard clinical types of observations. And it

wasn't just tremors.

There were a number of other things that were happening. I think they were -- I don't have the spread sheet here. Maybe --

DR. CORY-SLECHTA: But again, my question is were they subjectively assessed? Were they operationally defined measures of behavior that somebody systematically looked at or not?

DR. LI: They are not. But, you know, I have done both the FOB and also been study director for studies in which -- the standard clinical observations.

And I agree that the standard operating type of procedures with the FOB are superior. But I also believe that the clinical observations have been pretty good at being able to detect real effects that happened.

So usually, in fact, they won't cull the tremor unless they really see the tremor.

DR. CORY-SLECHTA: I think it is very difficult to see in a rodent.

DR. ROBERTS: For the record, the response was from Dr. Li.

Dr. Foster.

DR. FOSTER: I have a couple questions. I suppose it was from your summary of the 2003 two generation dietary study where you claim there is no effect on pup mortality in the presence of pretty significant cholinesterase inhibition.

DR. LI: Right.

DR. FOSTER: Would you then argue that the two have got nothing whatsoever to do with each other?

DR. LI: I don't think that we're saying that there is any mechanism relating cholinesterase inhibition with pup mortality. We're not saying that.

We're just saying that, unlike the DNT study, there is a very different pattern of effect here. And you can't say that it is exactly the same as what you are seeing in the DNT study.

DR. FOSTER: Do the registrants then have any extra data on comparing dietary versus gavage kinetics? Because it strikes me that the only way you can address this is you need to know what the dose to the target is. We haven't been presented with that. You have to take (ph)

it before assumption.

DR. LI: I'm not aware of data from the registrant on that. In fact --

DR. FOSTER: One other thing. When you looked at your delivered doses from your dietary studies, did you actually make any calculations for what that would have been during pregnancy?

Because it won't necessarily be what the adult females got.

DR. LI: Your question again is?

DR. FOSTER: What would be the delivered dose to a pregnant female?

DR. LI: From a dietary exposure? Because it changes.

DR. FOSTER: Right.

DR. LI: The doses that I think -- what we did is we just used the doses that EPA calculated. I think that's basically an average over a period of time.

But there is data likely on food consumption and then chemical concentration that you are able to do that. And so you will see that it changes over time.

And it is, in fact, like when the pups start getting into it, they can get actually a pretty large dose, milligram per kilogram per day.

DR. ROBERTS: I think Dr. Raffaele has something to add.

DR. RAFFAELE: I believe in that particular study they adjusted the parts per million dose to try and maintain a constant level of incorporation.

DR. FOSTER: That wasn't constant incorporation.

DR. RAFFAELE: It was not constant parts per million. It was to try and maintain a constant dose.

DR. FOSTER: Thank you.

DR. ROBERTS: Thank you, Dr. Raffaele. Dr. Chambers.

DR. CHAMBERS: Dr. Li, I was a little confused on your comment about the amount of dimethoate that's converted to omethoate. Would you mind repeating that?

DR. LI: I'm just looking at a standard metabolism study. I was looking at omethoate data. So the obvious question that came to my mind is how does that relate to dimethoate.

What they have is I would say about 90 percent of the administered dose comes out in the urine. And of that amount that comes -- and then omethoate is 1 to 5 percent of the administered dose.

So they know how much they give from radio activity to -- in the metabolism study. And then they are measuring how much omethoate is coming out in the urine. And so that's approximately 1 to 5 percent of the administered dimethoate dose.

DR. CHAMBERS: I have another question for somebody. Can somebody clarify how the cholinesterase assays were run and how the tissue samples were held and processed prior to the running?

MR. HAZELDON: As far as the cholinesterase analyses were concerned, they were conducted within the same laboratory.

The samples were taken and frozen down, then sampled -- then analyzed. I can't tell you how long they stayed in storage before they were analyzed. I don't have that information. But they were all done in the same lab.

DR. CHAMBERS: Was this like a clinical analyzer

that was used for calorimetric assay?

MR. HAZELDON: I will need to check my report for that information. I can't tell you which assay was used. But it was, if I remember correctly, it was the methodology that was advocated by EPA for this purpose.

DR. CHAMBERS: Do you have any idea how long the tissue margin sat before they are actually analyzed once they were thawed and ground up?

MR. HAZELDON: How long they sat?

DR. CHAMBERS: Yes, before the actual assay was run.

MR. HAZELDON: I don't know.

DR. ROBERTS: Dr. Reed.

DR. REED: This question may be for John.

This morning, maybe I heard it wrong, I thought what was presented on that dam Number 19 was that the dam was going down even before the pups were born and so forth.

And it was going from what is being called in the document as aggressive behavior into a sort of abnormal behavior.

What I'm sort of struggling with is when I read the document it says it displayed aggressive and restless behavior. The pups were observed to be thrown around and trampled and scattered.

Is that the dam that you were describing as abnormal behavior?

DR. DESESSO: I think so. Yes.

DR. REED: It doesn't seem to be consistent with the dam which is reduced in body weight and going down --

DR. DESESSO: I took out aggressive because aggressive sounded too subjective. But the dam was also losing weight.

DR. REED: I understand that. But it was trampling and scattering the pups.

DR. DESESSO: Yes.

DR. REED: I don't know who I should direct this question to. But could someone take me through the November 20th document called dimethoate key issues for the assessment of potential human health risk.

In table 5, which is in page 15, postnatal day 1 to 4, and looking at the first 3 groups, 1 C, 1 A, 1 B,

you have three out of three, two in one and twelve in five. Does it look like there is an increase or is there a sort of adjustment or way that you would interpret the data differently?

Postnatal date 1 to 4 on the first three groups, 1 C, 1 A, 1 B?

DR. DESESSO: What --

DR. REED: It appears that there is an increase. You probably went through this. I just didn't catch it. Would you consider this as not an increase of incidence or an increase of --

DR. DESESSO: Are you talking about where it says -- are you on the line that says postnatal day one to four?

DR. REED: Postnatal day 1 to 4, yes. Not 1 to 11, but 1 to 4.

DR. DESESSO: You have three deaths, two deaths, four deaths?

DR. REED: Table 5.

DR. DESESSO: Oh, I'm sorry.

The 12, that's the one that's removed. 3, 2,

12.

DR. REED: This one has A and B superscript on it. A would be not including the stillbirth. B would be -- oh, this one includes the pup --

DR. DESESSO: Yes. And then the one down below where it drops down to four is where that dam was removed.

DR. REED: But you don't have the one to four data. Right? Postnatal day 1 through 4?

DR. DESESSO: What --

DR. REED: 3 to 3, 2 to 1, 4 to 4 (ph) is what it is. Right?

DR. DESESSO: Yes.

DR. REED: Thank you.

DR. ROBERTS: Any other questions? Dr. Pope.

DR. POPE: I'm wondering about the time of peak reduction in cholinesterase activity in the pups versus the mom at GD 20.

DR. LI: What was your question?

DR. POPE: I want to know what the time course of inhibition and recovery in the pups versus the dam's brain is.

DR. LI: That time course was not measured.

DR. POPE: Do you think that could be important in determining which one is more sensitive?

DR. LI: Yes.

Well, actually, because we're talking about repeated doses, I guess the thought is that it is less important to determine the time of peak effect. However, to the extent possible, they try to use the information from peak effect in adults to -- I think they had discussions with the EPA on what the best time to set for the fetus.

Now, Keith -- there wasn't a specific experiment in which they trapped the cholinesterase inhibition over time for each of the periods. That was not done. So I can't produce data that says that this is the peak effect and this is how fast it went out.

But for the repeated exposures, we're thinking that the time of peak effect is less important. However, to the extent possible, there was an attempt to try to measure and take the brain cholinesterase at as close to the time of peak effect that was related to times when

there was peak clinical effects that were seen in adults.

I think there was some decision that maybe they would lag the time a little bit thinking that it might take a little more time to get through the placenta.

But in terms of hard core rigorous data to answer your original first question, there isn't data there.

DR. POPE: I'm thinking that with this particular compound there may be a very rapid peak in the fetal brain. And because it is synthesizing a lot of proteins, including cholinesterases you may see a very rapid recovery compared to the maternal brain.

DR. LI: Yes.

DR. POPE: So that time course may be very important regardless of whether you're doing repeated dosing or single dosing. There may be little spikes at each time point.

DR. LI: Yes. There was an attempt to try to get at that time of peak effect.

DR. POPE: Based on the mom.

DR. LI: Right.

DR. ROBERTS: Dr. Fischer.

DR. FISCHER: Dr. Li, I'm going to read you a sentence that you read to us from your talk. It is the one that says because adult brain cholinesterase inhibition from repeated exposures is the most sensitive endpoint, protection against it will protect against pup mortality.

So one question I have is or comment that you can respond to is that if I read this in a particular way, it sounds to me like you are saying that you know that cholinesterase inhibition is causing pup mortality.

DR. LI: No. That's not -- I'm sorry if that's what was concluded from that. We don't think we understand any kind of mechanism between cholinesterase inhibition and the pup mortality.

However, based on the BMD analysis and our other sort of weight of evidence analysis, cholinesterase inhibition in the dams is -- I mean in the adults is a more sensitive endpoint than pup mortality.

So if you make sure you protect against that lower level, then we believe you will protect against the

pup mortality. So if that sentence leads you to believe otherwise, then we need to rephrase that question.

DR. FISCHER: One more. In your reading, have other cholinesterase inhibitors besides this one caused pup mortality on a regular basis? In other words, I'm asking whether there are other cholinesterase inhibitors that show the same effect that you are seeing that we're seeing with pup mortality.

DR. LI: I think Kathleen might be in a better position to answer that question than I am.

DR. ROBERTS: Putting you on the spot, Dr. Raffaele.

DR. RAFFAELE: I think that someone had asked a similar question earlier. And the answer as best I can recall, and I have not looked at all the data for all the OPs, so I can't make a great generalization, but there is an increase in pup mortality in some of the reproductive toxicity studies with other OPs.

However, it generally occurs at doses considerably higher relative to the doses that cause cholinesterase inhibition than we saw in the dimethoate

studies.

DR. LI: And reproduction studies are often -- are frequently for pesticides dietary. So we also see this larger differential in the dimethoate reproduction studies. It is in the gavage study that they are coming out closer.

DR. ROBERTS: Dr. Harry.

DR. HARRY: Just one point for clarification. I know you said we had it, but everything has been focused on the pup mortality. That was the critical endpoint that -- that and the cholinesterase inhibition were the critical endpoints identified in the DNT study. Correct?

Were there any other adverse effects on any of the other endpoints within that study that you remember?

DR. ROBERTS: Dr. Raffaele.

DR. RAFFAELE: There were some other effects that were noted in the study that were discussed I think by Cheminova in one of their previous papers that we had not thought -- that we had resolved basically to the point where we didn't feel that we needed to bring them before the panel.

DR. LI: There are many different behaviors that were evaluated as Kathleen showed in the earlier slide. And I think the only effect that we thought was treatment related was decrease in activity from the FOB type of observations where they cross -- you count how many times they cross lines.

And in a three minute period, that occurred at postnatal day 21. And that's what we thought was treatment related effect.

DR. ROBERTS: Dr. Harry.

DR. HARRY: One more quick point of clarification now that you are reminding me. If I remember correctly, you saw it there, but it was not picked up when the automated motor activity was done.

DR. LI: Not at postnatal day 21.

DR. ROBERTS: Dr. Pessah, then Dr. Cory-Slechta.

DR. PESSAH: What was the dose at which you saw the decrease in that particular activity.

DR. LI: That was the 3 milligrams per kilogram, the highest dose.

I think we agree with EPA's assessment on the

motor activity. But I have to tell you it has been a while. We quickly -- we looked at that just to make sure we understood what was happening there. Because that's also important in understanding what might be happening with DNT both in utero and later.

And we quickly came to the conclusion that there really wasn't that much happening functionally and that the two critical effects were really this pup mortality and this cholinesterase inhibition.

So we very rapidly focused in on those two endpoints.

DR. ROBERTS: Dr. Cory-Slechta, then Dr. Collins then Dr. Francis.

DR. CORY-SLECHTA: You ran I guess a series of behavioral assays from the DNT when the animals were I think 60 or 65 days of age. I think it included things like learning and memory.

Can you tell me how the statistical analyses on those were done? It certainly appears when you look at the data, at least the group means, that there are differences, significant differences in the slopes across

time.

So my question was how those data were actually analyzed statistically.

DR. LI: I was looking at Joe, because he is the one that has really looked carefully at the --

That, we can get to you. They are going to look up the actual report and get that answer to you.

DR. ROBERTS: Dr. Collins.

DR. COLLINS: I guess the $64,000 question is what is causing the pup mortality. And having listened to Dr. DeSesso and reading his fine report here, he seems to indicate maternal toxicity -- maternal toxicity could certainly play a part.

But usually when you have maternal toxicity and you have it to a point where you are starting to see death in the pups, you start seeing some real effects in gestational weight and in the lactational weight.

Now, if you look at the good old DNT study, which I guess we have killed to death here, you really don't see drastic effects at the 3 -- here you have the 89 pupparoos (ph) dying and three total litters gone and .5,

whatever.

And you look at the gestation weights. You don't see much. You look at the lactation weights. Unless my eyesight is going, and it probably is. You look at food consumption. You don't see much there.

Really, it is a mystery of what really is going on here. The maternal toxicity -- now, in the cross fostering study, you have some effects on the lactation, but not really a lot that would really, you know -- going to kill a lot of animals, going to start killing pups.

DR. DESESSO: The thing is we were careful to use the word maternal influence. I know it is kind of like dancing -- it sounds like semantics. But we didn't think we had enough data to come out and say these animals -- it was toxic.

But there do seem to be some alterations in the way that the dam interacts with her pups. I don't know what else to call it. It does seem to show up repeatedly.

It is not something -- it would be great if we could say, look, they all lost weight. We wouldn't be having this meeting if we had that. We don't. It is a

puzzle.

And it is obviously not simple. And I couldn't come up with a great experiment that would give us the answer either right off the bat. We could probably come up with a very complicated one.

DR. KEEN: If I could add on slightly to what John's answer was. We don't have as much information on effects of maternal toxicity for one of a better term on the lactation side as on gestational side.

I think that's what is leading to a lack of what are the sort of indicators we should follow. If one looks at the human literature, then there are a number of situations, high stress, high trauma, that can result in full to partial lactation failure that is often times consistent also with the mother either not initiating breast feeding.

And yet, these are in the absence of any overt signs. You don't see marked reductions in the mother's weight, you don't see marked reductions in food intake. It is clearly a stress phenomenon.

Regrettably what is lacking here is good

measurements --

DR. COLLINS: These are stressed animals.

DR. KEEN: Exactly. But you can have stress without necessarily having marked changes in body weight or food intake.

My suspicion would be that this could be the mechanism by what's occurring here. I think the key observation is otherwise one has to envision something which has not yet been defined.

Since the direct exposure of the pups didn't result in direct toxicity, I think that's an observation that's fairly significant.

DR. ROBERTS: Dr. Francis.

DR. FRANCIS: This is actually a very -- somewhat of a change of direction. But you talked about the 1990 one generation study. And at what level was the pup mortality? You said in terms of low, medium, high. I guess this is for Abby Li. But you didn't say what the doses actually were.

DR. LI: It was 5.8 and 7.5 milligrams per kilogram per day. That's based on the EPA's calculation

converting from the parts per million.

DR. FRANCIS: So the dose at which there was increased mortality in the pups would have been 7.5? I'm asking? Or both?

DR. LI: It is the two highest dose.

DR. FRANCIS: Oh, so at 5.8 also?

DR. LI: Right. And that at both of those doses, as well as the one below that, there was substantial cholinesterase inhibition. And the two gen repro, the dose estimate is a bit of an estimate compared to like the direct dosing where you know exactly what you are giving to the dam.

DR. FRANCIS: Right.

DR. ROBERTS: Anymore questions? Dr. Foster.

DR. FOSTER: I have one more. And this is for you, John, I suppose. I'm still grappling with these pups that were stillborn, died within the first six hours.

Comparing your table 4 to 5, it looks like there were about twice as many that were in the 6 mg compared to the control.

DR. DESESSO: Yeah. And the reason we went back

and looked at all of the, what you would call, seed litters for this group, that's to say you've got -- we had more litters than we actually put into the cross fostering study, or the contract lab did, because you have to have that bigger population, and when we looked at that, because we were looking for -- we were saying, is there something going on here, when we did that, all of a sudden the numbers seemed to wash away.

It was like -- it just happened that the animals that had the pup deaths in them were the ones that wound up getting incorporated into the cross fostering study.

Had it been some of the ones that were left behind, we wouldn't have seen that change.

I think it's a statistical artifact. But I don't know. It is something -- we told you the whole story so you can make up your own mind. We're not trying to hide this.

It is something that we grappled with as well. And it wasn't until we went back and looked at that group more closely to find out what was going on that we discovered that, gee, there is like another six or seven

litters that had no deaths in it and then when you do proportion of pup deaths, they are essentially the same as it is in the controls.

I mean, they are slightly higher, but they are not -- it's 1.8 versus 2.4 isn't enough to write home about.

It is --

DR. FOSTER: It's kind of perplex --

DR. DESESSO: You guys can arm wrestle over this for the next couple days too.

DR. FOSTER: Probably will.

DR. DESESSO: You probably will.

DR. ROBERTS: Dr. Pessah.

DR. PESSAH: One of the things that this data doesn't tell me is is there a narrow window susceptibility between postnatal day 1 and postnatal day 4, because you haven't treated directly pups at that age.

So there may be a narrow window of susceptibility that hasn't been examined here. There may be transfer through the milk and trying to find the data in terms of --

DR. DESESSO: First of all, the likelihood that much is transferred through the milk I think is relatively small. There is very -- as Dr. Raffaele mentioned, there is very little inhibition, cholinesterase inhibition in the first four to eight days.

DR. PESSAH: We already said that cholinesterase inhibition doesn't correlate with lethality.

DR. DESESSO: Okay. And although we don't have the studies yet, the only thing we are suggesting was to see whether or not how much, if any, gets into the milk. We don't know that.

That would be a good thing to know. That would tell us --

DR. PESSAH: Even a very small amount if there is a critical target that we haven't yet identified could, in fact, be responsible and you may not pick it up as a really big transfer through maternal milk.

DR. FRANCIS: It also could be a non cholinesterase inhibiting metabolite of dimethoate, a breakdown product in theory.

DR. DESESSO: In theory. That would have to be

the omethoate. That's the only one that's been identified.

You know, we don't do a lot of direct dosing to pups in the first four days because you get a lot of problems when you do that.

They get stressed out. They die or do other things. And so it is a tough question. The thing is we don't have data to answer the question at this point.

I can't say that you are wrong. And my gut tells me that you may be wrong. But then again, as you point out, you have this business about the very rapid protein synthesis in pups, which is real.

And it is one of these things where nobody has a lot of background data on this. It is like the old adage. The more we learn, the less we know. And we're just getting into this field.

The more we get into it, it is really complicated.

DR. KEEN: If I could add to that. It may either help or hurt depending on one's perspective. But given the fact that it is within hours after the cross

fostering is done that you see the first deaths, then often times you are beginning to envisage (ph) a metabolite which is yet to be defined that would really have an effect on a process that is about as fast as anything I can think of.

Now, that doesn't disapprove it, but it does begin to stretch I think the credibility -- it is hard to envisage (ph). I can't think of another example of a compound going through milk that has this sort of rapid effect.

And we do a reasonable amount of work with looking at milk toxins and different compounds that transfer -- and I can't think of any corollary.

DR. ROBERTS: Dr. Lein.

DR. LEIN: That actually raises an interesting question for me, Dr. Keen, which is do you guys have a distribution set of data that shows when during PN 1 and 4 most of those crossed pups are starting to die?

Are they mostly within a couple hours of being crossed as you just implied?

DR. KEEN: No, I'm sorry. I didn't mean to

imply that. What I was saying is that some did die within a very short time period.

If I recall, I don't have the data set memorized, but it was not all on day 2, day 3 or day 4. They were rather well distributed. But a subset of them died very quickly.

So I was really kind of focusing on that for my comment.

DR. ROBERTS: Dr. Harry.

DR. HARRY: To bring it back to the maternal question, is there any data or evidence out there that suggests that this compound exposure can alter any hormonal levels that we know would be related to maternal behavior?

Anybody that knows the data set.

DR. LI: We're not aware of the data from the data set we saw. And I think the data set -- we pretty much presented to you the data set that we looked at.

But the folks from Cheminova are saying that they don't have any data on its effect on hormonal effects.

DR. ROBERTS: Response by Dr. Li.

Dr. Raffaele, did you want to add something?

DR. RAFFAELE: Yes. We don't have any data on hormone levels. There was, however, in that second reproductive toxicity study some impact on male reproductive organs.

I can't remember off the top of my head exactly what it was. But that may or may not.

DR. ROBERTS: Dr. Francis.

DR. FRANCIS: This is on the question of the pups that died quickly. I sort of have been assuming that the maternal influence would be something in the line of neglect, scattering the litter, kicking the pups around.

How quickly would that act? Unless the female is actively destroying them, they wouldn't at least -- my experience is more with mice than with rats, but I don't think they die that fast from neglect.

DR. KEEN: If it was simple neglect, I would agree. Because, in fact, you can do up to slightly the different type of experiment, those things called pup in the cups.

So when you are doing artificial formula feeding, we can take pups away that are newborn and we might take them away for six hours and then go ahead and feed them at that point. So simply being ignored for that time period would not necessarily cause death.

On the other hand, in those studies which have primarily been done with mice, if you don't have good thermal control, you can lose them in about 12 hours.

So it is actually fairly fast by simply not having good temperature control.

DR. ROBERTS: Dr. Harry.

DR. HARRY: In any of the animals that you lost, did you by any chance take any of the morban (ph) animals that you knew were going to die and look at the enzyme level in those?

DR. LI: No.

DR. KEEN: Carl Keen answering a collective no from behind me.

DR. LI: We have an answer about the statistics for the learning and memory test if we could provide that for Dr. Cory-Slechta.

DR. ROBERTS: Thank you, Dr. Li.

DR. REISS: I didn't do the statistics myself, but I just took a look at the study report. And they used a standard multiple comparison test called Williams Test, which is very similar to Dunet's (ph) Test.

If the variance -- they would do a check for the homogeneity of the variances. And if they weren't homogeneous, meaning the variances between the groups weren't equal, they would log transform the data and then repeat the test.

But I would caution you about against comparing the group means from the data that at the brief look I just took at it. The coefficients of variance are all in the order of 40 to 50 percent, meaning there is a high degree of variance in the data. I think there is 10 animals, maybe.

You would just statistically you would expect a high degree of natural variance between the mean responses.

DR. ROBERTS: Dr. Cory-Slechta.

DR. CORY-SLECHTA: But those are repeated

measures across time. So I think what you are telling me, if I'm correct, is that each data point was analyzed as if it were an independent piece of data when, in fact, those are measures across time?

DR. REISS: I think they -- no, I can't -- I think the different days you are talking about, I think they would analyze them all separately, yes.

DR. LI: They are two different time points. I understand what you are saying, Debbie. Many laboratories do not analyze it like a repeated measures across time.

DR. CORY-SLECHTA: I disagree with that. The standard in the field is to measure repeated measures, to use repeated measures.

DR. LI: I understand. I absolutely agree with you. I'm saying that's why I understand what you are saying. But in this particular study, I would -- I didn't look at the statistics section, but I would bet they did it on each day separately, just from what I have seen of statistics for these studies.

DR. CORY-SLECHTA: Because doing each day separately, I would agree with you. But it is really the

slope across time that is of interest. And you are doing something very different with repeated measures looking at that than you are doing a day by day analysis.

DR. LI: I think I would feel more comfortable if we had more time points, but when you just have two, it's still --

DR. CORY-SLECHTA: I think as I remember, you had at least 9 data points because you had 3 trials per day for at least 3 sections.

DR. LI: You are saying the learning curve.

DR. CORY-SLECHTA: Yes.

DR. LI: Okay. Right.

DR. ROBERTS: Are there any other questions?

DR. RAFFAELE: Dr. Chambers had asked about the assay method for the cholinesterase. It was the calorimetric (ph) assay. And there is information in the DERs which I can show you later if you want to see one in terms of procedural information --

DR. CHAMBERS: I read that, I think, in the materials we were sent earlier. But what I don't get a sense of is how long the samples may have sat on ice or

room temperature and all before they were assayed. And that was really kind of critical information.

DR. RAFFAELE: I don't have that information either.

DR. ROBERTS: Now seeing no questions, further questions, let me thank the group from Cheminova, Dr. Li, Dr. Reiss, Dr. Gaylor, Dr. DeSesso, Dr. Keen for your presentations and patience in answering many of our questions. It was very helpful.

Obviously, you have spent a lot of time sorting through these data trying to make sense of them, and we appreciate you taking the time to present your analyses for us.

We have next on our list of public presenters Dr. Jennifer Sass. Welcome, Dr. Sass.

DR. SASS: Thank you. I'm Jennifer Sass. I'm a scientist in the Health Program with the Natural Resources Defense Council. It is an environmental nonprofit group. We're located here in Washington, D.C.

My background is as a laboratory scientist. I was for about 10 years in the lab, mainly toxicology,

molecular biology, a lot of tissue culture, a lot of neural and developmental biology.

So I'm going to be presenting a much shorter presentation on what the public interest, essentially community, feels about the dimethoate risk assessment as it is presented here.

I want to thank the Scientific Advisory Panel, first of all, for allowing the opportunity for the public to speak and also for coming together for really what is going to be a long and arduous week.

I can tell you that I will be here with you the whole week. You will see me a few times. And I do appreciate your time. It's a labor of love. I really do appreciate it.

I also want to thank the last presenters for their time. They, obviously, put a lot of work into their presentation. It was really was very enlightening I think for all of us. For me, certainly.

I'm going to start out trying to stay with your charge questions, although I know later I fall off a bit. But, first, you were asked to comment on the impact of

maternal neglect and maternal toxicity on pup mortality from the DNT study.

Our feeling is that the observed pup death, which has been acknowledged by both the previous presenters and the EPA, is a valid endpoint. It is supported at the high doses by the cross fostering study.

And we feel that the maternal neglect or maternal toxicity or whatever might be contributing to the pup death is -- may be a real effect of dimethoate toxicity.

The fact that it might be maternal effects that are contributing to pup death should not be negated or disregarded. It should be considered as part of the toxicity.

How it is part of the toxicity I think nobody knows. I don't think we really have a good sense of what is going on. I think that's important to keep in mind too.

The second question was to comment on the evidence from the cross fostering study that supports or refutes the observed pup mortality from the main DNT

study.

The cross fostering study data do support the observation of pup mortality in the DNT study in the high dose group.

I think the problem that's been discussed already is that the cross fostering study fails to repeat the DNT study design for the mid and low dose groups.

So I actually think it is just not very informative in those low dose groups. Therefore, I think it is not very informative to the key question, which is what is happening in terms of elevated pup mortality or increased pup death incidents in the mid and low dose groups.

The data from the cross fostering study are of limited use given that the doses tested did not include the lowest dose, the .1 or the medium dose, the .5 used in the DNT study. It is hard to apply the information.

We support the EPA conclusion that the cross fostering study confirms pup mortality at the higher doses suggesting that dimethoate does contribute to increased pup death.

Whether this is pre or postnatal maternal exposure, I don't think anybody can answer clearly, but certainly we know that it is treatment related. And that's what is important.

The endpoint of pup death is a valid endpoint and should not be disregarded if it may be due to maternal exposure, maternal negligence or some other hypothesis which is inadequately tested at this point with the data available to us.

Question 1.3, comment on the use of the brain cholinesterase inhibition as an endpoint for dimethoate acute and chronic risk assessment, and would it be adequately protective.

You were asked to evaluate whether cholinesterase inhibition in the brain would protect against pup death and presumably any other dimethoate adverse effects.

That second part I think has been inadequately charged in the charge questions and inadequately discussed. And that's the point that I would like to bring forth, although there was some discussion of it at

the beginning in the last round of questions.

We feel that the cholinesterase inhibition study was actually not designed to measure noncholinergic effects or the neural developmental endpoints.

The DNT study, the developmental neuro toxicity study was designed to do that. And so these endpoints must be quantitatively included in any assessment, that is, the DNT endpoints, including what may be non cholinergic toxicity.

In the DNT study, there was 23 to 24 dams per dose that were treated. And then the offspring from each litter were treated. It was a very robust study. It was designed to look at developmental, neuro toxic effects by design, whether those are cholinergic or not.

In the cholinesterase studies, as far as I understand, and I listened all morning and I might still not understand this, but my understanding is that the dams were treated, but actually only 2 pups, a male and a female from each of only eight to 10 dams were actually subjected to the treatment regime.

In other words, although the dams underwent

treatment, there was far less pups. Far less pups. There were actually, as far as I understand, hundreds more rat pups in the DNT study.

And therefore, it is more robust and more adequately designed to examine the effects on pup mortality and the more subtle developmental endpoints that were revealed in the study and that I'll be discussing.

If there is a correction, somebody should let me know. It is not going to affect the rest of my slides, but I don't want it to go in the docket incorrectly.

The DNT study suggests adverse motor effects at the lowest dose tested. On postnatal day seventeen, males showed a dose dependent increase in horizontal motor activity of 43, 65 and 122 percent compared to controls.

It was dose dependent. But it was not statistically significant. But when you look at the variability, these are the standard deviations seen under postnatal seventeen here, here is 0, the .1, the .5 and the 3 milligram per kilogram per day doses for the DNT study.

And here you see that at zero they counted motor

activity data, they counted cage floor activity, total activity counts for each session. So this is number of counts. Number of activities.

And there is 171 in the control group. But the center deviation is 147, which is almost the same as the number of counts.

In the .1, there is 244. It increases by 43 percent. But the standard deviation is 231. In the medium dose, .5, it is 281 counts of activity. It increased 65 percent compared to controls. But the standard deviation is 405. 379 counts in the high dose. But the standard deviation is 407.

So the standard deviations in these two doses are actually higher than the actual number of counts. And in the lowest dose, it is almost as high.

So to rely on statistical significance reminds me of that joke where the drunk relies on a lamp post the way some people rely on statistics which is for support and not for illumination.

NRDC supports the EPA conclusions, that despite the lack of statistical significance, this is a quote from

the EPA DER, the data evaluation report on this DNT study, "Despite the lack of statistical significance, it is reasonable to consider the dose dependent increases on postnatal day 17, the ones I just showed you at .5 and 3, that's 65 percent and 122 percent increases, as treatment related."

The DNT study also suggests, we feel, adverse rearing effects at the lowest dose tested.

This is in appendix 2 of table 9 in your handouts as long as the appendix numbers for this SAP is the same as the appendix numbers for the SAP for this subject that was originally scheduled in July. Because that's the appendix.

On postnatal day 17, male rearing activity was increased, again non statistically, by 104 percent, 154 percent and 98 percent for all doses. That's in dose dependent order.

Mean rearing scores for females on the same, postnatal day 17, were decreased at all doses, 58, 43 and 90. But only the high dose effects attained statistical significance.

They used a P value of .01, not .05 here. And I want you to note the very high variability, again making statistical significance, I would suggest, pretty close to impossible to obtain.

Here in the postnatal day seventeen, this is for rearing, total activity counts for rearing at the control group there was 12 counts. But the standard deviation is 16.

At the lowest dose tested, there was 25, but the standard deviation is 38. Middle dose, 31 counts, but the standard deviation is 68, double the actual number of counts. And at the highest dose, 24 counts, but the standard deviation is 29.

For the females, same, postnatal day seventeen. For the controls, 46 counts, but the standard deviation is 56. 19 counts at the lowest dose, but the standard deviation is 20. 26 counts in the mid, but the standard deviation is 23. And 4 and a half counts in the high dose, but the standard deviation is eight.

That's the first one that actually attained statistical significance at the .01 P value that they

used.

Again, I would suggest that one should read this with a more open mind than simple reliance on statistical significance when the variability is so high.

The conclusions from the DNT data, EPA reports non statistical adverse effects in pups for both rearing and motor activity at postnatal day seventeen males and females at the lowest dose .1, increased total pup death at .5, and increased litters with pup death at the highest --

They actually broke out, which I thought was interesting, pups per litter and then counting number of litters that were affected.

EPA is concluding that there is an acute BMDL 10, the 10 percent benchmark dose of the lowest level, the low endpoint -- the lower level for brain cholinesterase inhibition of 1.3.

That's what is in your handouts. And when they calculate all these different ones for pups in 2 -- what they present in their conclusions is they say there is a range. It ranges between 1.3 and 2. But if you break it

out, the 1.3 is for the pups and the 2 is for the adults.

For chronic benchmark dosing for the 10 percent level for brain, it was .2. And for pup mortality it was .6. So the argument that EPA is making to and that the registrant is supporting is that if you use brain cholinesterase, you will be protecting against pup death.

That might be. But there were no calculations at all given for the motor effects. And the BMDL 10, which is still a 10 percent level, it is still a 10 percent level of effect, will not be protective from the motor effects in pups that are seen at the lowest dose tested, which was .1.

Our recommendations are that we support the use of the benchmark dose analysis as a more comprehensive review of the available data in general.

We support the use of the cholinesterase inhibition data in general for developing benchmark doses. They are not inherently, I think, any weaker or any less reliable than the other data.

We recommend that the FQPA factor be used to account for chronic, potentially non cholinergic effects

on motor function and neuro behavioral reported in pups at the lowest dose tested from the DNT study.

At the moment EPA is using no FQPA factor. In other words, it's reduced to one. So there will be no adjustment factor.

The assumption that EPA is making now is that pups and adults are equally sensitive and that pups are not more sensitive.

But the reports of motor activity, adverse effects on motor activity in the pups were at levels where the adults were not reported to have any effects, although, there was, I think, no reporting on the adults in that area.

And the levels that were reported in the pups from the DNT study on behavior were below the BMDL 10 derived from the cholinesterase data. Possibly because they are not cholinesterase dependent effects. I don't know. I don't think we know.

But certainly it suggests if the mortality data is not tracking well with the cholinesterase data, then maybe that's not cholinergic effects either.

So we suggest certainly the FQPA factor is in order here. And we recommend that EPA also include an uncertainty factor to account for the effects that by definition occur at a BMDL 10. These are 10 percent effect levels. This is not a no effect level.

Thank you for your time.

DR. ROBERTS: Thank you, Dr. Sass. Let me ask the panel if they have any questions. Dr. Cory-Slechta.

DR. CORY-SLECHTA: I have a question of the registrants about the loco-motor activity data. In that experimental design, are the same groups of pups the same actual pups used for the different days of testing.

Again, is that repeated testing across time, and were the data analyzed by repeated measures. Because when you look at that data even with the variability attached to it, I would think you would get an interaction in a time by dose statistical analysis.

And was that carried out. Because it would show up at a lower dose.

DR. ROSS: Joe Ross.

DR. ROBERTS: Your affiliation?

DR. ROSS: Ross Tox Services. Those were the same pups.

DR. CORY-SLECHTA: Then my second question is was statistical analysis carried out using repeated measures analysis since they are not independent assessments at each time point as you know because it's the same sample.

DR. ROSS: That's a question for the statisticians.

DR. LI: I do not believe that the data was analyzed using the repeated measures analysis for 13, 17 and 21.

What is also interesting about that pattern is that I think you see an increase at the 17. And in a lot of the data when you look at 13, 17 and 21, 13 you have a lower level of activity, and then 17, in many cases, you get a higher level of activity. It is part of the --

DR. ROSS: Developmental pattern.

DR. LI: Yes, developmental pattern. And in fact, that's what actually happens in the dosed groups. It didn't happen in the control.

DR. ROSS: Overall, really, the data are just simply so variable that it is really difficult to make much of them.

And I think if you would look down, you might say, well, maybe the equipment or the personnel or the laboratory was somehow deficient in the conduct of the studies.

But you can go to the postnatal day 60 or 59, I think it is 59 there, and when they did the observations -- I'm sorry, the recordings in the adults, and the coefficients of variation were, I think, around 25 percent there, they are much, much lower.

And if you go back into the raw data, you see things in the data here that are consistent kind of as, for those of us who measure pups, that we see frequently.

If you look for an hour, some pups will be active, then they will be a lot less active, and then suddenly there will be a burst of behavior at the end. There just simply is variability. It makes analysis of the data very difficult.

DR. CORY-SLECHTA: I fully appreciate that,

which is why I never use total counts to describe loco-motor activity data because they have no meaning in terms of time.

But my other question would be how long exactly was the test session for these.

DR. ROSS: It was about an hour. It was close to an hour. It might have been -- it was an hour.

DR. CORY-SLECHTA: But again, did you look at those data? You can break all of those data out by time. Because I assume it was done in an automated fashion.

DR. LI: There was habituation curves. We tried to look and see whether there was any pattern effect there.

DR. CORY-SLECHTA: But again, unless you do it by repeated measures, looking at it on a day by day basis, all you have done is inflate the variability to some extent. And you don't have the time interaction factor to look at.

I guess, then, I would go back to my other question. Why didn't you see an ontogeny of loco-motor activity in controls.

DR. LI: I have seen enough data now. In the studies where they saw the ontogeny, and this is sort of getting off of dimethoate, they did the test every single day.

I think it was -- yes, the Rupert (ph) studies. Are you familiar with the Rupert studies? They saw this person, but they were testing every day.

One of the things that was stated -- because sometimes we see it and sometimes we don't, was that if -- it was an oral communication. That if you didn't test them every day, like if you were testing them every three days, sometimes you didn't get that pattern.

So I just think that the test is such that we're going to get that variability -- because that's what Rupert saw as well. She didn't see. They don't get that peak all the time when you just do every 3 or 4 days.

DR. ROBERTS: Dr. Portier.

DR. PORTIER: We're having a discussion over here about -- the statisticians, about how would we analyze this data. One of the things I noticed as we looked at this charge, the first thing we said this is not

even normal data; it is count data.

It is going to be distributed -- a log transform, so is the analysis done with a log transformation?

I gave him an opening here.

DR. REISS: This a question I can answer. Yes. We did the analysis by log transform and the results were the same statistically. But one of the unique things when you look at it in a log transform basis a lot of those patterns sort of disappear and the curves look very flat.

Also, we also looked at historical control data from the laboratory. Because we had such a high variability -- you know, there's cautions in doing that, but we felt justified because we had such a high variability.

When you look at the -- when you add in the historical control data from the laboratory, you also see a dampening of the patterns you see there and the conclusions about statistical significance remain.

DR. PORTIER: A follow-up question from Dr.

Roberts.

DR. ROBERTS: I have another -- a follow-up question.

It is always dangerous to pick out one or two things in a big set of a lot of statistical analyses to say these are the two things that are significant. And I don't have the appendix in front of me, but I have a feeling you ran a lot of tests and a lot of them were not significant and these were significant. And if that's the case, then I'm not that worried about this.

DR. REISS: And these weren't significant actually, except there was one at the high dose that was significant, I believe. But at the low and the mid dose they weren't significant whether or not you log transformed it or not, whether or not you added the historical control data or not.

DR. LI: As a behaviorist, we consider that data to be important. But having looked at it, we are just not seeing a pattern that we believe is treatment related at the PND 17, postnatal day seventeen. We did see -- think we saw something at the PND-21, postnatal day 21 at the

highest dose.

DR. REISS: There is an effect on motor activity via observation on day 21 in both males and females in the 3 milligram per kilogram dose, and having looked at that in cholinesterase inhibitions, both kind of the level, the amount of the decrease and the cholinesterase inhibitions in those pups are very consistent with data that Dr. Chambers has reported, Karen Chambers in 2000, about a 40 percent decrease in cholinesterase activity and you get a decrease in motor activity via observation.

Very consistent, and we think that's a treatment related effect, it makes perfect sense.

DR. ROBERTS: Did you have a follow-up?

DR. HEERINGA: Just continuing a little bit on this. I agree with Dr. Portier, too, that when I look at these count data I think in terms of a poisson-type model, poisson-type progression models. And that is an old model that I think has really come back with some of the generalized linear model methods.

Even the growth curve type analyses are mixed models that Dr. Cory-Slechta is referring to -- can be fit

to these poisson data with things like packlon (ph) mix and others too. I think I would take a look at this data at some point using a poisson-based likelihood as opposed to the log transform on a normal base likelihood.

DR. LI: I just wanted to add. I don't want to dismiss also Ken's comment about looking at patterns of effect. Like for example, the rearing. It is one of maybe 32 measures that you make on an FOB and you really do -- I mean, the fact that EPA guidelines for neurotoxicity risk assessment cautions everyone that this is exactly the problem.

You look for that, many observations. But what I wanted to do is just make sure we weren't dismissing important behaviors. And we consider all this data to be very important. And so with respect to the rearing, you have to look at that in the context of everything else that is going on.

And so again, we conclude that there is a treatment related effect on behavior at PND-21, postnatal day 21 at the highest dose.

DR. ROBERTS: For the record, the response was

from Dr. Li. Dr. MacDonald and then Dr. Portier.

DR. MACDONALD: I think I would go even further than Steve Heeringa's suggestion, because what you are describing, it seems to be something complicated but certainly real.

You've got an in-homogeneous group, you've got active pups and you've got inactive pups, so you have to start analyzing it more as a mixture of the proportion active and then how active the active ones are.

I think that's where you are losing the significance. It seems that the averages you present are also very enticing, that they are suggesting strong effects and the standard deviations are coming from having mixed up too much together when you do the analysis.

DR. LI: The other thing we try to do is look also at what happens at the first 10 minutes of that motor activity, because sometimes towards the end there is just a lot more variability. So we really have tried to look at the data in many different ways.

I just wanted to say that I have been involved in a couple of meetings in which we're trying to wrestle

with these questions of how to analyze behavioral data on DNT study. So what was done by the registrant, by the contract lab is something that's done very commonly by many laboratories that are conducting these studies.

We are trying to address this issue on a more generic issue basis on how to address the fact that the design is repeated measures. And there are other statistical ways to look at this. And so I acknowledge and recognize the comments that are made.

But we have looked at the data in spite of that and there is just a lot of variability and our assessment is that there is an effect at PND-21, postnatal day 21 at the highest dose.

DR. ROBERTS: Again, response by Dr. Li.

Dr. Portier, did you have a question?

DR. PORTIER: No, just a last comment.

You are absolutely right when you are doing a lot of testing and then you find one or two things significant, I think what Dr. Reiss said was the right answer. It also confirms what we generally believe would happen that raises it to a significant effect rather just

another test, one of the 5 percent that we make a mistake on.

DR. REISS: Yes, the fact that this one statistically significant result occurred at the high led us to, I think --

DR. PORTIER: Well, not only that, but that it fits with the literature as we know it. There are many uncertainties here, but that's something that we can make sense of.

DR. ROBERTS: Dr. Cory-Slechta.

DR. CORY-SLECHTA: Let me go back to this number of tasks. When you run these as repeated measures analyses as variance, all of those would have been collapsed into one statistical analysis. You would have had a potential to look at an interaction term which is time.

So yes, you may only get one or two when you do multiple tests like that, but what that negates is the fact that you do have overall either main effects or interactions that you are not looking at --

DR. ROBERTS: Dr. Harry.

DR. CORY-SLECHTA: -- if you are going to stay on the statistics.

DR. ROBERTS: Go ahead, Dr. Portier.

DR. PORTIER: I was just going to come in -- I don't think it all totally collapses. Yeah, you can get a slightly better measure of the underlying variability by taking into account the repeated measures effect. In fact, you may actually find a few things more significant in the general picture.

I don't know how much data they really have to do a big repeated measures analysis, although you have like six or seven time points. Right?

DR. CORY-SLECHTA: The kind of data you are looking at right there, the standard in the published literature is to use repeated measures analyses of variance date.

DR. PORTIER: I don't disagree.

DR. CORY-SLECHTA: The sample sizes are consistent with what is typically in the literature. I wouldn't see the why this would be any different.

DR. PORTIER: I don't disagree. I just don't

think it is probably going to change the results all that much.

DR. LI: Having done analysis on behavioral data and trying to do it the right way using repeated measures analysis, you still have lots of different endpoints. I mean, even with the FOB it is harder to collapse all that data together.

There is like 32 different endpoints, then there is the auditory study, then there is learning and memory. So there is really a lot of behavioral data. The fundamental point of looking for patterns of effect is really important. We try also not to just say that if you see the behavior, but, no -- you ignore it.

So when you are looking for patterns of effect, you don't want to ignore behavioral effects that happen in the absence of other things, but in this particular case there was a really nice pattern of effect that made us call what we thought might have been an equivocal effect -- a real effect. We had a disagreement about it. So we're --

DR. ROSS: Joe Ross again.

The effect on motor activity by observation on day 21 was statistically significant. So I mean it wasn't marginal, I mean if was in both sexes.

DR. ROBERTS: Dr. Harry.

DR. HARRY: To go back to the issue that actually started us on this motor activity question, which was bringing up here on doing it postnatal day 17. If my memory serves me right, which it could be wrong, the logic behind trying to look at motor activity in such young animals, which was questionable, about how well we could really measure that to start off with when it was talked about putting it into the guidelines in those discussions, the logic was going back to that developmental ontogeny, which really is a day 15, day 16, day 17, day 18, day 19. It is not a day 13, day 17 and day 22.

And it is a fine window around that day 17 of where you are expected to see this hyperactivity linked to the circuitry that's warming within the brain and then coming back down.

So if you are just looking at day 17, you would expect you would get a massive amount of variability.

So I raise the question that if you don't do it, to look at this whole -- like every day for a week repeated measures, same animal, it can be different animals too and it seems to still hold up -- that do you truly have a valid independent point by looking at day 17 for motor activity when you know you are going to get this much variability.

You could be off by 12 hours in that animal and you may be different. So that's one question. And so I raise that as a concern because it is being an isolated endpoint that is raising issue of whether that is a critical point that you should come back to.

And I raise the question to ask should we consider whether that's really a valid measure given the developmental timepoint of those animals, any animal, any rodent. Anyway, we would expect such horrible variability.

DR. LI: Basically, we have a guideline. That guideline was developed -- having done this experiment, it may sound easy to do motor activity 13, 17 and 21, but the idea of doing 13, 14, 15, 16, 17, 18, 19 -- the pups are

not all born at the same time.

I think that -- that's really the right way to do the experiment, but the guideline asked for 13, 17 and 21. When you look back on what it is the basis for that, it is exactly as you are saying to try to measure that.

When I really started seeing all the variability of that data I went back and really looked very carefully at the Rupert paper and I found that quote that said, Oh, and by the way, if you only measure the behavior every 3 days, you don't get that increase curve, but sometimes you do in other papers.

So I agree with you that I don't know how valid a measure the 13, 17, 21 is on the ontogeny of the motor activity. But the other confounder is the design of the study now requires us to dose the pups from PND-11, postnatal day 11 to 21.

So in those experiments that Rupert did, they purposely avoided dosing during the period of 13 - 17, you know all that period where they were trying to look for the ontogeny. They stopped before that so they wouldn't confound the acute effects on that ontogeny.

So now with the design the way it is, it is really difficult to make any kind of interpretation of the data. We just want to look very carefully to see is there an effect. And I don't know if that answered your question. Does that answer your question?

DR. ROBERTS: Follow up by Dr. Harry.

DR. HARRY: Just as a point of information. I was more concerned about trying to bring it up since that issue had been brought up as a point raised for concern about how comfortable are we with that data happening at that day.

For information, if you go back to the maternal component, we have been doing similar studies and doing them in mice.

What is interesting is what we are finding is that you can shift this developmental ontogeny of motor activity either a day before or a day after of where it peaks.

And it is totally dependent upon and correlated with the activation state of the mom and how she is simply interacting with the pups, whether you have a mouse that's

very hyper and not necessarily doing optimal maternal behavior. But still none of the marks that you would have what you guys were detecting that you would start increasing the variability because you are starting to shift the day of when that peak is.

So this could be influenced by maternal components.

DR. ROBERTS: Dr. Sass, you stimulated quite a bit of discussion with your last point.

DR. ROBERTS: Are there any questions for Dr. Sass before we complete her comment?

Thank you very much, Dr. Sass, for your comments and the presentation.

Before we close the public comments session, I will extend the offer to -- if there is anyone else in the audience who would like to make a comment to the panel on this topic?

DR. ABRAHAM: My name is Dr. Abraham Tobia (ph). I'm with Bio CropScience. Today I'm not representing Bio CropScience, I'm representing Crop Life America. Normally, Angelina Duggan (ph) would be here, but,

unfortunately, she is home nursing a broken foot so I am substituting for her. So don't make any comments on my looks or anything else like that.

But we have asked Barbara Neal, Dr. Barbara Neal to give a presentation that we feel will help this panel in the discussions they have had today. This is an industry perspective on the benchmark dosing and the ten-percent effect levels that we're looking at. I'm going to ask Dr. Barbara Neal from the Weinberg Group to give this presentation.

DR. ROBERTS: Welcome. Have a seat. Please introduce yourself for the record. You don't have slides, right?

DR. NEAL: I have slides, but I have some copies.

It's a very brief comment particularly looking at the benchmark dose ten for brain cholinesterase inhibition. Point out this is based on the detectable effect level and first slide.

Looking at cholinesterase as a continuous endpoint, not a quantal endpoint, there is a wide range of

background variability in control animals. And there is obviously no practical method for looking at measurement of individual background brain cholinesterase data.

So what I looked at is multiple historical data sets which provide a basis for looking at the cholinesterase variability. I think this addresses an early question by one of the panel members. Basically, looking at multiple data sets, I wanted to thank Bayer and Mahkteshim for providing control date.

These don't include any of the Cheminova data, but they include data from Sprague Dawley and from Wistar rats and looking at a number of different studies by both gavage and the diet, percent co-variance, looking at -- basically 5 to 10 percent range average.

There are some obvious highly variable measurements. These are 8 to 10 animal data sets in these control groups.

Next slide is Wistar rat data showing a very similar pattern. And this I think portrays the ontogeny of the brain cholinesterase through the fetus PND-4, PND-11, 17, 21 and adult. And the variability is fairly

consistent across ages and between laboratories.

These laboratories all, I believe, used a modified calorimetric (ph) method for cholinesterase. I know in the case of two of the laboratories the brains were flash frozen as soon as they were taken, put into nitrogen basically to preserve them and analyzed immediately on following the tissue.

Going to the next slide, these show further sets of Sprague Dawley rat data. Go on -- these are Wistar rat data, again, looking at the same endpoint. And although the scales are slightly different, each laboratory reported in somewhat different units, there is a very consistent pattern of ontogeny between the two rat strains.

This doesn't speak to unique susceptibility of one strain over the other, but it certainly suggests that a similar response might be anticipated between strains. And these are adult data.

I think the basic message is that 10 percent were selected, not that this is 10 percent of a population that is impacted, but it is a 10 percent is the detectable

change from control.

And the last slide, basically, I wanted to just step back and look -- sort of 6,000 foot view of some of the other elements in the DNT study design that are inherently conservative and may be thought of as protective.

We're using an animal model that's basically very immature at birth both in terms of its capability for detoxification, it partially models in utero human, but without the maternal protective metabolic capability.

Many of these studies including the dimethoate studies use gavage administration, which gives you a good measure of exposure, but not necessarily a good model for environmental or dietary exposures.

And the studies essentially require that the high dose produce toxicity. So you know that you are dosing to overwhelm metabolic capacity and you may introduce confounders due to maternal toxicity.

That's basically the extent of my comments, that we're providing a conservative point of departure for risk assessment using this endpoint.

Thank you.

DR. ROBERTS: Thank you. Are there any questions from panel members on the presentation?

Don't see any, thank you very much for your comments.

Are there any other members of the audience that would like to address the panel?

Before I close the public comment portion of the agenda, let me remind you that once the public comment portion of the agenda is closed, that's the last opportunity for -- to address the panel or make points.

Okay. I think we've covered that aspect. The public comment period then is -- for this meeting is then closed. Let's take a break for 15 minutes or so and then reconvene and we can perhaps tackle the first question.

(Thereupon, a recess was taken.)

DR. ROBERTS: For the Panel, in case you are curious, there is a new handout that has appeared during the break. This is a color version of a handout you received earlier today.

So some of the figures may be easier to see in

color. So this color version was produced for your benefit, but it is a duplicate of one that you saw in black and white earlier today.

Well, we have had quite a bit of -- we've had some very interesting and informative presentations. Already some lively question and answer discussions sessions. I think we're primed and ready to go to tackle the first question.

So let me ask, then, the Agency to pose the first question for the panel, please.

DR. RAFFAELE: Question 1.1.

Please comment on the information available for dimethoate which characterizes the underlying cause of pup mortality in the dimethoate DNT study and the degree to which this information can be used to determine the impact of maternal neglect, maternal toxicity on pup mortality.

Dr. Roberts asked me if I could give a short version of this question which is basically, I would summarize it as -- is the information available to us, does it enable us to figure out what the cause of the pup

mortality is?

DR. ROBERTS: Dr. Reed. What is your opinion on this?

DR. REED: I will start out. I'm sure our group of people will have lots of other comments on this. But let me start by saying that I think the cross fostering state is very useful in my mind when I review it, to allow me to closely differentiating sort of the relative contribution from the postnatal maternal effect to the prenatal fetal exposure effect.

Of some endpoints -- not just the pup death, the issue that we're sort of grappling with right now. Cross fostering state in general I think can be used to investigate or make the differentiation. I think in this case it was very useful that I think it is clear in my mind that the postnatal maternal effect is there.

The question is whether we can, based on these studies, the cross fostering and the DNT and the range finding and so forth, the entire database, whether in my mind I could attribute the entire pup death effect to the postnatal maternal effect. I think that's what we wanted

to figure out.

My answer is no, it isn't clear that there is no contribution of the prenatal part of the exposure to the pup death, especially during the early part of the postnatal period.

One thing I think is very clear that many of the controversies related to this is in the data analysis how, especially how we include or exclude certain data sets and specifically the day number 19 or litter 126 and also litter 139 or day 139.

We have seen a lot of presentations and the reasons -- and I read about the document, and I'm not ready to dismiss the counting of it in data analysis. I appreciate data analysis within that data set, but I didn't feel it warrants the description of the dam or warrants the dismissal of the completely -- and I understand that with that, whether include or exclude whatever great influence on the final conclusion.

I also have a comment about drawing conclusions or whether decided we would go forward benchmark dose response or not in terms of data set. But I also feel

like that determination should not just be based on statistical significance.

My point of argument is that especially with something as adverse as death, I think we need to treat that data more carefully in that if there is an indication of increases -- incidence, we do need to look at it.

It doesn't just confirm statistical comparison between the treatment group and the control. I think all three dose groups in this case in the cross fostering state 03 and 6 should be looked at as a group. And if there is an increase with the dose or indication treatment increase, we need to look at it more carefully.

The example being in, I think table 9 in page 24, of November 1st, a document from Cheminova, there is indication -- and I brought this out earlier on, there is an indication that the pre cross fostering data indicated there might be some increase of pup death before cross fostering.

And just simplistically, if I take a look data set and plug it into benchmark dose model, it actually gives me an indication or a statistical analysis saying

that there is increase dose. Earlier on I think there was a comment or response to my question saying that yes, but the response is very low, below 5 percent.

But I think we're looking at the absolute value of response and not percentage of response up and above the control when the control is still there.

So I would encourage the Agency to take a look at that data set and any data set related to that from DNT study or range finding or any other studies for that particular endpoint, early pup death.

In terms of not being willing to dismiss the contribution of prenatal exposure, I was also looking at the table 4 of the November 1st document where it shows the 00 group has I think one increase with no milk in pups. But there is 10 and 7 on the 03 and 06 groups and I'm not sure if that is sort of nothing.

There are several endpoints I think or data sets that cause me to have doubt about whether it could dismiss the prenatal contribution.

Talking about behavioral endpoints, I also feel that we need sort of a closer analysis on that mostly

because I'm not quite sure if it is not a part of the entire evidence of prenatal exposure, not just pup death, but behavior effects.

The last thing is earlier on we talked a little bit about whether pup death is an endpoint for OPs in general and I know the Agency has not looked at that. I cannot look at all the -- it was about a year ago, I looked at all the OPs and actually it is not an uncommon end.

I think it was brought on early on to say it wasn't an uncommon endpoint, but it was a higher dose. I'm convinced of that when I look at the data and so I would encourage -- my comment is I would encourage the Agency to go and look at all the data that you have. Not just at DNT study, but from the study to see if there is indeed some pattern in this particular endpoint.

And perhaps this analysis could benefit from the larger database, but also the larger database analysis could benefit from the analysis from this particular case.

DR. ROBERTS: Dr. Collins, is there enough information to determine the cause of the pup mortality?

DR. COLLINS: Well, quite frankly there really isn't. We certainly have increases in pup mortality in three different studies as we have reviewed them. The DNT study, the range finding study and as we have already been over the infamous cross fostering study.

Now, the question is what causes the effect; is it maternal toxicity? Well, I have already discussed earlier that if you look at maternal toxicity, certainly it could play a role. But certainly in all these studies there certainly isn't a huge effect in maternal body weight.

There is some effect in lactation weight on some of these -- in some of these studies, but really not a huge, drastic amount.

Now, does the cholinesterase play a part? In most of the time where you see pup death, you also see inhibition of brain cholinesterase.

However, in some cases you have the inhibition, but you don't have the large amount of death especially in the dietary studies. So you have a third possibility. Is there -- I think Dr. Foster brought it up or somebody

brought it up of an unknown metabolite in there, something else that is actioning or do you have stress on these animals that also is causing the problem.

We don't know, and I think quite frankly in looking at these studies, and I think we do get some confirmatory information of the cross fostering, and it's been mentioned before, we certainly should have had a control. I think it is sort of a, shall we say a flawed -- for that reason I think it is sort of a flawed study.

What I would like to see, and I hate to recommend additional work because I'm sure the registrant loves to hear that, but I would like to see a teratology study done according to the new guidelines where you dose them all the way to day 20.

And maybe let them live, let them give birth and let them live during this period of time, the six hours or 12 hours or whatever and see what you have over a period of at least those levels that we looked at.

The teratology studies that you had done in the past were done according to the old guidelines, which were done to the genesis.

This may give you some definitive action of whether or not gestational effect has had a large effect or not. But to really have scientific proof of what is going on here, you don't have it. We can postulate here to until the cows come home, in my view.

DR. ROBERTS: Dr. Cory-Slechta, got enough information?

DR. CORY-SLECHTA: Thank you. I would agree with my two predecessors in terms of conclusion, largely for the reasons that they have already described.

I'm troubled also in particular in the cross fostering study by the fact that we don't have a cross foster control group and how the stress of cross might influence mortality ultimately would be the real baseline here.

I think the other thing that probably was obvious during the questioning, at least from my point of view, I certainly believe there can be maternal toxicity and changes in maternal behavior, but I don't think the cross fostering study was really designed to answer that.

I think people made a valiant attempt to go back afterwards and see if they could somehow relate it to that, but those kinds of observations can be done and maybe should be done and people certainly do maternal toxicity kinds of evaluations, but it needs to be done in a quantitative, operationally defined manner so we can look at the data and really make some judgments.

So I think again as I said before, I agree with the first two speakers in terms of the conclusion. I just don't see enough data there to rule out -- to come to the conclusion that there is no role -- that the role is really maternal negligent or toxicity.

DR. ROBERTS: Dr. Foster.

DR. FOSTER: I will just say I agree with everything that has gone before. It just strikes me that we really don't have any mechanistic information that tells us why these pups have died. I think we can't ascribe all the pup death just to a postnatal maternal neglect component.

And so we have to, by default, because we don't have the information in front of us to say it is likely to

be from both gestational and postnatal exposure that causes the death.

I mean, again, you know, I already discussed with Tom about what the study would be to try to narrow down whether we really did have a gestational impact. This sort of thing where we're seeing these differences again between dietary and gavage exposure really cries out for some connects to help us know -- you know, dose the target and that kind of thing.

I know that's not a regulatory study or requirement, but I think it really does cry out -- that's an important component we don't have yet to try and meld all these studies together.

I think I had one other comment, because I've now heard it like four times and that was around gavage is not a suitable route of exposure for risk assessment.

And that reminds me of the broccoli study. And if you think about the pesticide that goes on broccoli and then you are trying to work out what human exposure is going to be, how many times a day do we eat broccoli? Probably only once and probably only in one small meal.

So it may be as opposed to trickle feeding it totally through the night, a gavage exposure might be more representative of human dietary exposure.

DR. ROBERTS: Dr. Harry, are you going to go against the other group?

DR. HARRY: Yes. I'm sorry.

First of all, as the question was reworded, is different than the question that's up there. And the question reworded to say --

DR. ROBERTS: Clarified.

DR. HARRY: You want me to clarify?

DR. ROBERTS: No, I said the question was clarified.

DR. HARRY: Well, on that question about what was the cause of the mortality, we have no idea. The studies were not designed to identify cause of mortality.

However, if you look at what the initial question asked, it asked is there enough evidence to say that the maternal influence, not toxicity, because there are things other than body weight changes that can

indicate maternal -- altered maternal behavior, is there evidence there to say that it could account for a number of mortalities that were being seen?

And I think the data is there to say do not discount the cross fostering study. I'm fine with throwing out animals that you know are invalid to look at. You know, if they are sick.

You know that they were sick and it was a control animal that got thrown out on that one, or something is happening within there. Or keep the animal, but cluster that data. And then when you come in and look at the data, you look at when you remove it.

Having done these types of studies, it is just a way that you are going to have to be open to looking at it. I think that when you were talking about doing a teratology study, I would come back and say that I think the effort that was made of looking at the pup mortality between what day 1 to 4 in all of those groups that were not used in the cross fostering, but to give you in sizes up to 71 litters, that that is a data set that should be looked at.

Because I think it gave a lot of information back about whether there would be a gestational effect or not. And we have only seen that in one presentation. So I think it is data that really needs to be considered since it is new in front of us.

So based upon my comment -- based upon the initial question, I think the supplemental information provided by -- supplemental information provided today by the presenters has raised a number and addressed a number of the critical issues about whether maternal toxicity could come into here. Trying to do a cross foster study.

If you were trying to answer all questions is a heroic effort. What they did was a very focused thing and I don't see where the cross fostered study for the specific question that was being asked is invalid by not having a control foster group. You did your control; you did your high dose. You did a full cross foster, you looked at the comparisons there. That is not an accurate way of doing a cross foster study to look at a specific question. It doesn't get everything. But it's a

fine -- it is an okay design. It is not an invalid design where I think the data is creditable.

I think the data presented raises the question that the maternal behavior was significantly inferential on the pup mortality. Whether you want to look at that to say that dosing to the mom had shifted the dynamics such that it is a toxicity to the animal and then it comes all back around to being an evaluation for toxicity on the offspring is a very different question.

But I think that the point has been laid out very well. The maternal toxicity is a true factor in here and that the pup mortality may not be your most critical endpoint, which is one of the other questions asked.

DR. ROBERTS: Dr. Riviere.

DR. RIVIERE: I've not really much to add to any of this. I think that there is definitely a maternal effect.

The only point I have notes on here is that I'm wondering if some of these effects are due to the large litter sizes that just happen to show up in the study and if that was an initial stressor that brought out some type

of a behavioral effect from the mother, something affecting the raising of the pups.

As far as the cross fostering study to detect this, I think because of the effect that we're seeing it would have been nice to have a cross fostering control.

Again, you could not anticipate what your results would have been to begin with. But that might have helped clarify some of this.

I don't think an answer, in the short version of this question, I don't think we know what is causing that toxicity and I think that's pretty clear. The lack of correlation with the cholinesterase inhibitions and the other studies is -- maybe it is not as spread as what apparently were historic studies, but these are relatively -- that raises a concern that we just don't know what is going on.

So no, I don't think that data can provide that answer. I agree pretty much with the initial commenters.

DR. ROBERTS: Let me ask if there are other members of the panel that want to provide input? Dr. Francis?

DR. FRANCIS: I don't know if this is -- it doesn't directly address the question asked, but the point has been made several times that the pups were dosed directly on days 11 through 20 and there were no deaths even though the dose there and the cholinesterase inhibition was greater than during the lacteal period when they were only getting rather small doses, apparently, measured by cholinesterase inhibition from the dam.

But I think that that is not really -- it is not really a deciding factor in a question of developmental toxicology because we know that there are windows of susceptibility.

And the fact that a high dose later on in adolescence so to speak, of the mouse -- of the rat does not cause toxicity, does not mean that an earlier lower dose could not have caused developmental effects. It's not directly on this question, but it does have to be kept in mind.

DR. ROBERTS: Dr. Chambers.

DR. CHAMBERS: I would agree very much with what Dr. Harry said but for somewhat different reasons. One

thing that needs to be considered, I think, with this compound, which I don't honestly know very much about itself, is that it is a dimethoate phosphate or it's an OOS compound or an OO compound.

The only one I really have any experience with is methylparthine (ph), but if the cholinesterase behaves -- the inhibited cholinesterase behave with this compound as it does with methylparathine, it recovers very quickly.

The half life of inhibited cholinesterase or dimethoate compound is a matter of couple hours as opposed to several days with the dimethoate compounds, so it is very reasonable to think that the dams that are dosed with a dimethyl compound like this are probably experiencing very high cholinesterase inhibition, that it recovers very quickly in a matter of few hours.

The reasons I asked about the behavior, if that was something that waned during the course of the day, the reason I asked about how the cholinesterase samples were handled, whether they sat or were allowed to recover for a little while, is that our experience with methylparthine

certainly is that the cholinesterase, if it is not assayed by a direct assay or a continuous assay very quickly it recovers on the spot sitting on ice.

I really think the dams could have experienced much higher cholinesterase levels which could have led to a very hyperactive variant behavior that could have affected the pups. So I think it is very reasonable to think that the maternal influence was fairly high, possibly due to cholinesterase inhibition that was transient from this compound.

DR. ROBERTS: Other comments? Dr. Harry?

DR. HARRY: Only because you had recommendations of a potential design for a cross foster study as in having controls is why I would say this. I think the only way to address all the questions that you would end up coming up with about what was there would be to have to do a completely randomized, split-litter cross foster design.

So you could really determine whether it was the individual litter, the individual mom, the individual levels in that mom at the time and whether it was maternal, prenatal or postnatal exposure.

That's a rather complicated design, and I'm not sure that given what we have on the activity levels that it is really something that really would be needed to be recommended.

DR. ROBERTS: Dr. Pope?

DR. POPE: I would like to follow up with Dr. Chambers' comment regarding the rapid inhibition and recovery following dimethyl compound. That's the point -- kind of the point I was trying to make as far as the peak time of inhibition and recovery with a chemical like this.

I think it is an important issue when you are talking about cholinesterase inhibition and relative sensitivity of the dam versus the pup and critical effect.

The second point that as far as the dietary versus gavage dosing, this same principle is in there. You've got animals that are just continuously eating a small amount and their cholinesterase inhibition is peaking and kind of equilibrating versus the gavage dosing where it is going up and down. And so I think the rate of inhibition is often important with cholinesterase inhibitors, not just the degree of

inhibition. This could be parlaying into the differential effects of the gavage versus dietary.

DR. ROBERTS: Thank you, Dr. Pope.

I think what I have heard from the panel, and the panel can please correct me if I'm wrong, is that there seems to be agreement that the information are not adequate to determine the cause of mortality in the pups.

There seems to be a difference of opinion to which the information available clearly demonstrates an association of a maternal influence in the effect. Is that a fair summary?

The panel can -- appears to be willing to suggest it is important to resolve this issue about maternal versus lactational versus in utero effects, the panel seems willing to contribute ideas for how such a study would be designed. We can do that in the minutes for this meeting, if that is something that would be of interest to the Agency.

That's not especially of interest to the Agency, I see so --

DR. PERFETTI: It may be of interest to the

registrants, but --

DR. ROBERTS: May or may not be interested in suggestions on what I'm sure would be a fairly expensive and -- so they may not be wild about receiving recommendations on those.

So we may not need to put those in our minutes. But if the registrant is interested, I'm sure we can -- members of the panel as individuals can provide them with some advice on that topic.

Is there anything -- let me ask, then, the Agency, is the response from the panel on this question reasonably clear so far? Is there a related follow-up question you would like to ask?

DR. RAFFAELE: Yes, I believe they have answered our question.

DR. ROBERTS: Would you like to pose the second question?

DR. PERFETTI: I think my team here would prefer to start tomorrow morning, Dr. Roberts.

DR. ROBERTS: In that event, we'll plan on taking up the remainder of the questions tomorrow morning.

We'll adjourn early today. We will reconvene at 8:30 tomorrow morning to take up the rest of the questions.

Again, I would like to thank the Agency, its scientists for their useful informative presentations and dialogue as well as our public commenters. I look forward to some further dialogue on these issues beginning tomorrow morning.

This session for today is adjourned we'll see everyone tomorrow.

(Whereupon, the meeting recessed at 4:15 p.m.)

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I, Frances M. Freeman, Stenotype Reporter, do hereby certify that the foregoing proceedings were reported by me in stenotypy, transcribed under my direction and are a verbatim record of the proceedings had.

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FRANCES M. FREEMAN

I N V O I C E

FRANCES M. FREEMAN

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